Kinetic biased signaling: towards a system biology definition of drugs selectivity

Romain Yvinec

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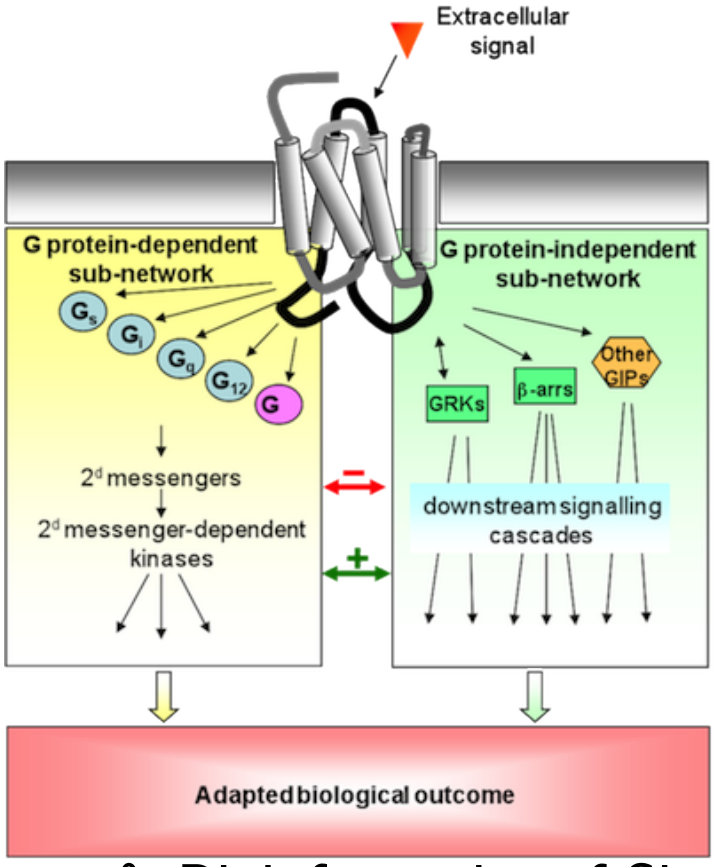
BIOS, INRAE Tours

BIOS group



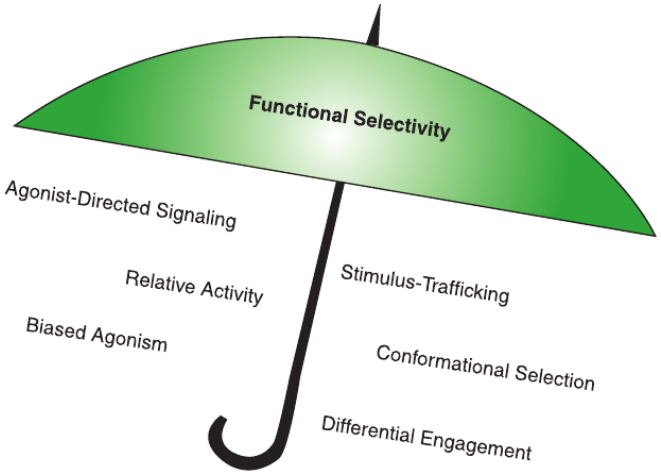
INRAE Tours, Physiologie de la Reproduction et des Comportements

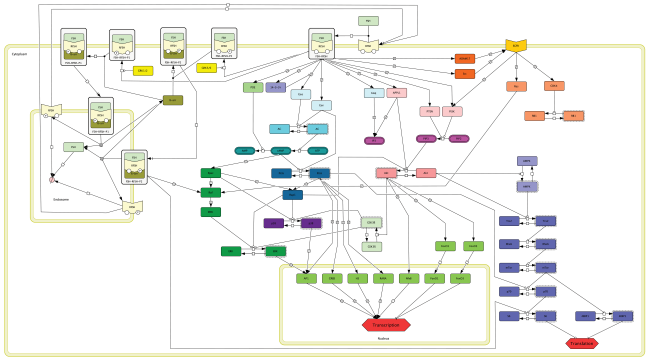
Biology & Bioinformatics of Signaling Systems : multidisciplinary approaches, linking biology, mathematics and information technology to experimental biology, in order to decipher the intracellular effects induced by reproductive hormones through the activation of their cognate receptors.

Functional selectivity, biased signaling

What is Drugs Selectivity ?

- Several reaction pathways are generally associated to a given receptor, and lead to various cell response.





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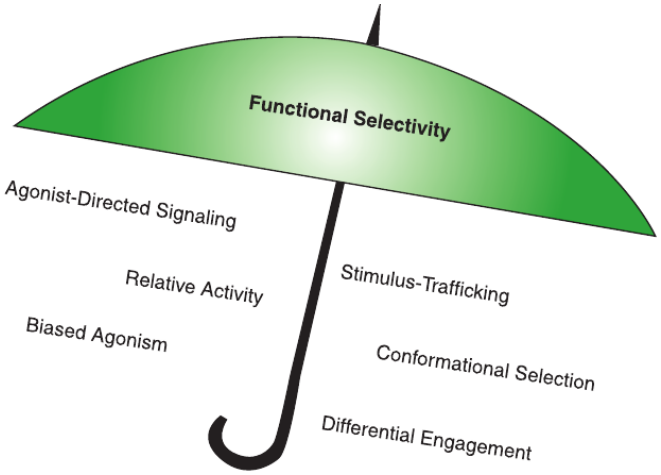
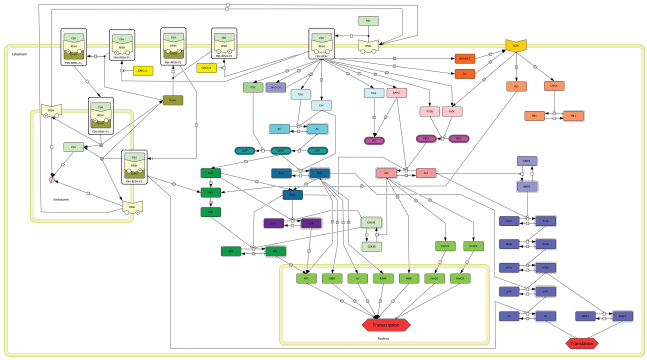
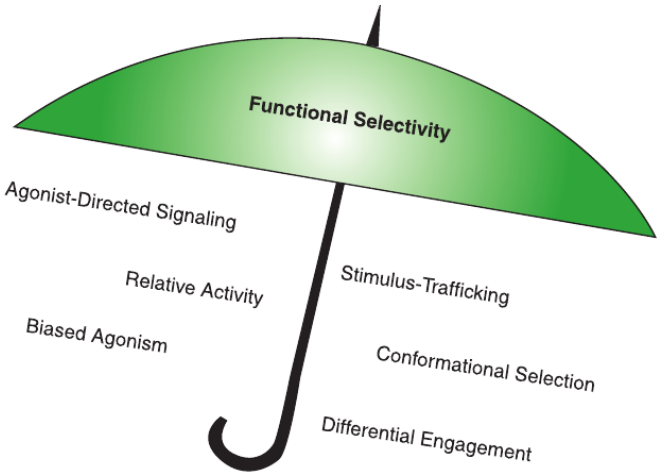


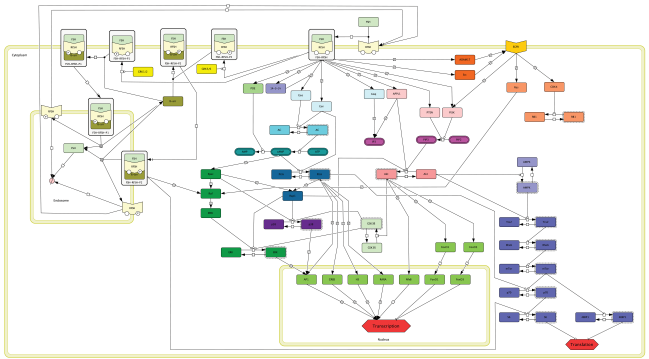


Functional selectivity, biased signaling

What is Drugs Selectivity ?

- Several reaction pathways are generally associated to a given receptor, and lead to various cell response.
- Differential activation of those reaction pathways, that differs between (natural or synthetic) ligand
- **Drugs Selectivity** = Ligand-dependent selectivity for certain signal transduction pathways at one given receptor





Key concept in pharmacology

- Drugs Selectivity (or Biased Signaling) is a key concept to be distinguish from
  - Partial or full agonist.
  - Antagonist, inverse agonist.
  - Affinity (K_d), potency (EC_{50}), efficacy (E_{max}).

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  - Affinity (K_d), potency (EC_{50}), efficacy (E_{max}).
- ◇ A bias might be **context-dependent** (cell type, physiological state, etc.)
- ◇ Biased agonism is becoming a **major tool in drug discovery**.

$\Rightarrow$ Candidate screening requires to accurately quantify bias.

Key concept in pharmacology

- ◇ Drugs Selectivity (or Biased Signaling) is a key concept to be distinguish from
  - Partial or full agonist.
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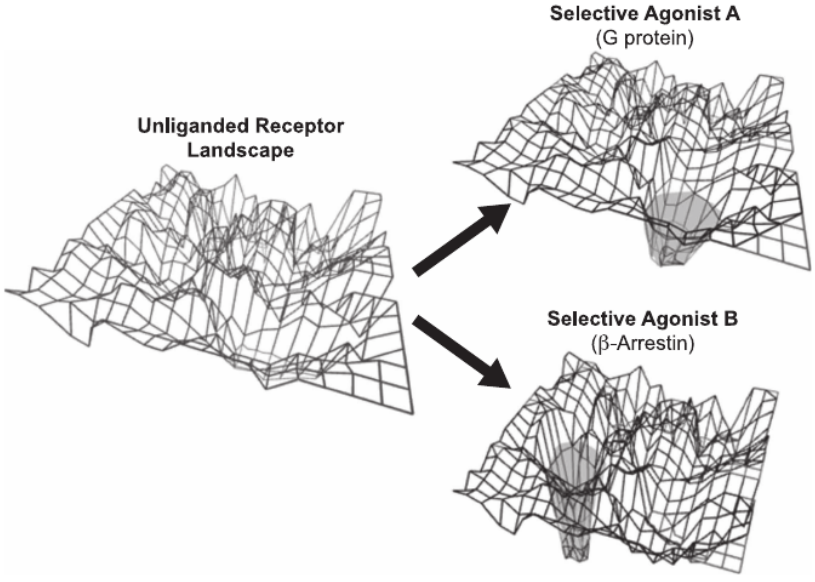
  - Affinity (K_d), potency (EC_{50}), efficacy (E_{max}).
- ◇ A bias might be **context-dependent** (cell type, physiological state, etc.)
- ◇ Biased agonism is also a powerful tool to **challenge our knowledge of signaling systems**.

$\Rightarrow$ "Perturbation" experiments with different biased ligands.

Theoretical foundation

A receptor may adopt several spatial conformations, each of which has different activation pathway profiles.

Conformational selectivity = Ligand-specific modification of the energetic landscape, changing affinities and efficacies of signaling patways.



Kenakin, *J Pharmacol Exp Ther* (2011)

Theoretical foundation

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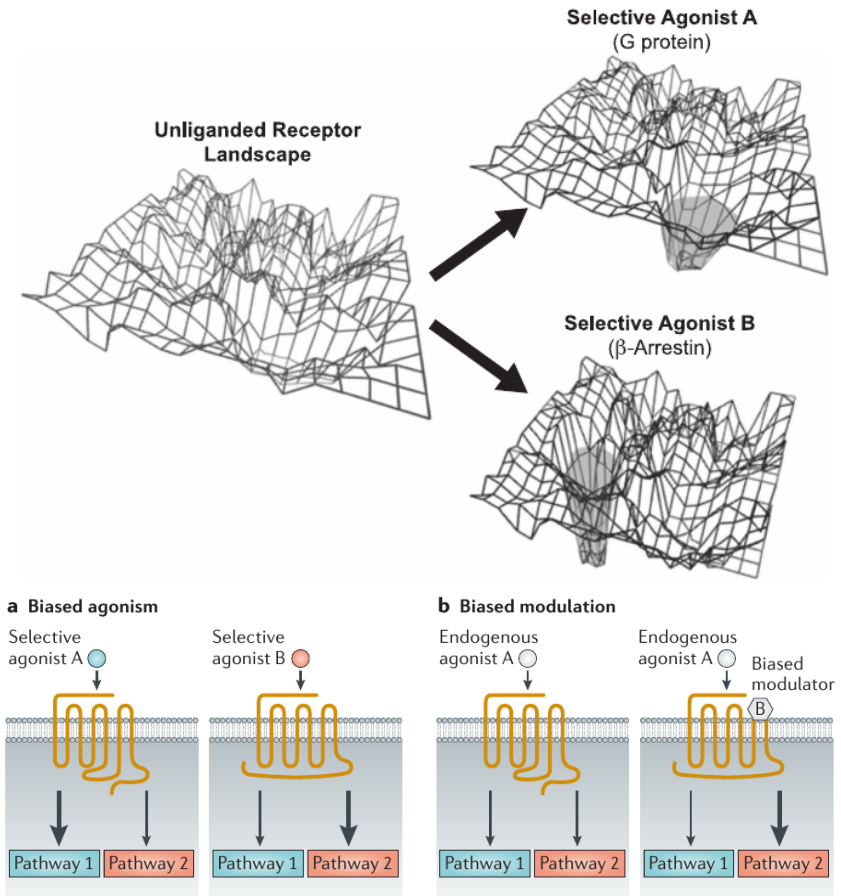
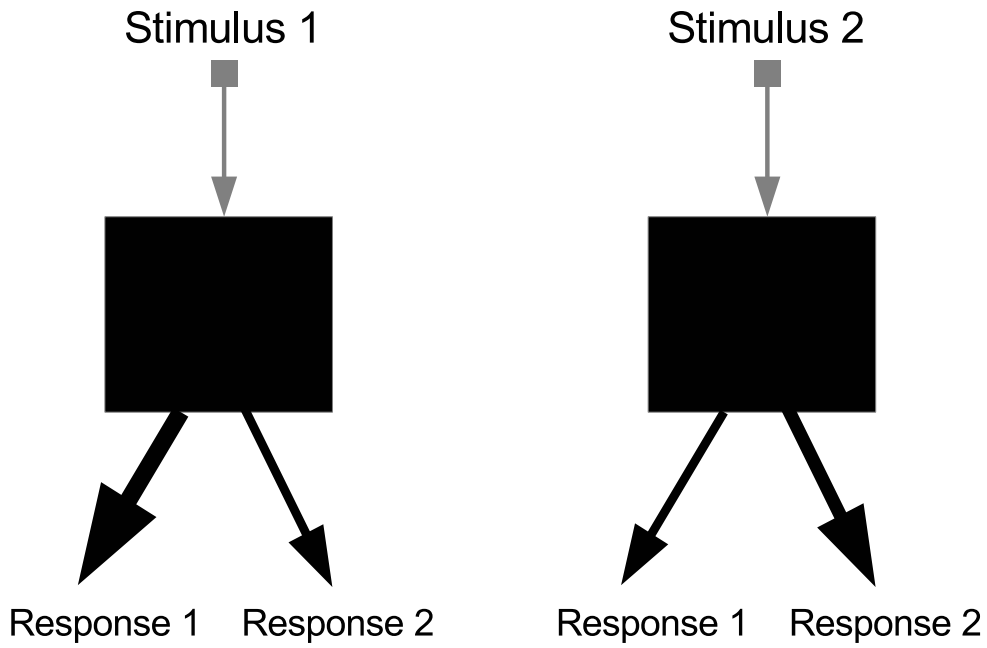
Similar concept : modulating bias



Kenakin and Christopoulos, *Nat. Rev. Drug Discov.* (2013)

Minimal setting

To speak about signaling bias, one necessarily needs **two** ligands and **two** responses, in a **same** cellular context.



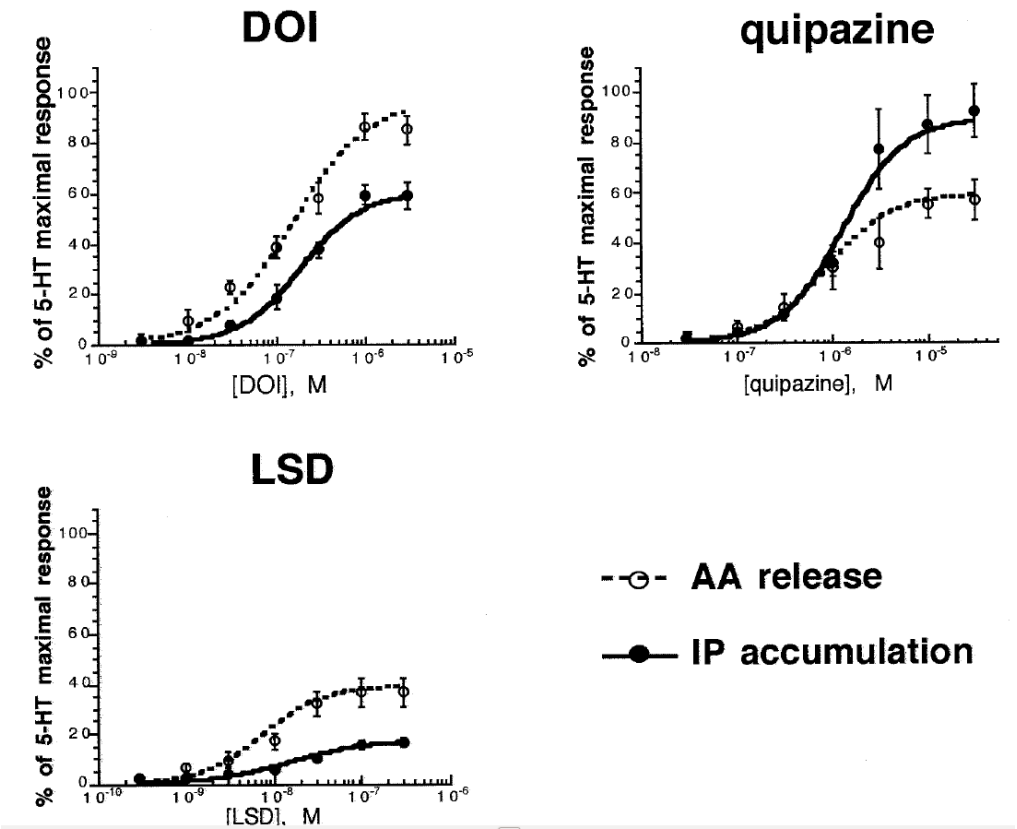
$\Rightarrow$ We always compare *a ligand with respect to a reference one.*

Outline

Serotonine receptor 5 – HT_{2C}

- Quipazine is biaised towards *PI* accumulation with respect to *AA* production, *compared to the reference agonist DOI*.
- *LSD* is not biased.

Berg et al., *Mol. Pharmacol.* (1998)



Serotonine receptor 5 – HT_{2C}

- Quipazine is biaised towards *PI* accumulation with respect to *AA* production, *compared to the reference agonist DOI*.
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$\Rightarrow$ Bias due to an E_{max} difference.

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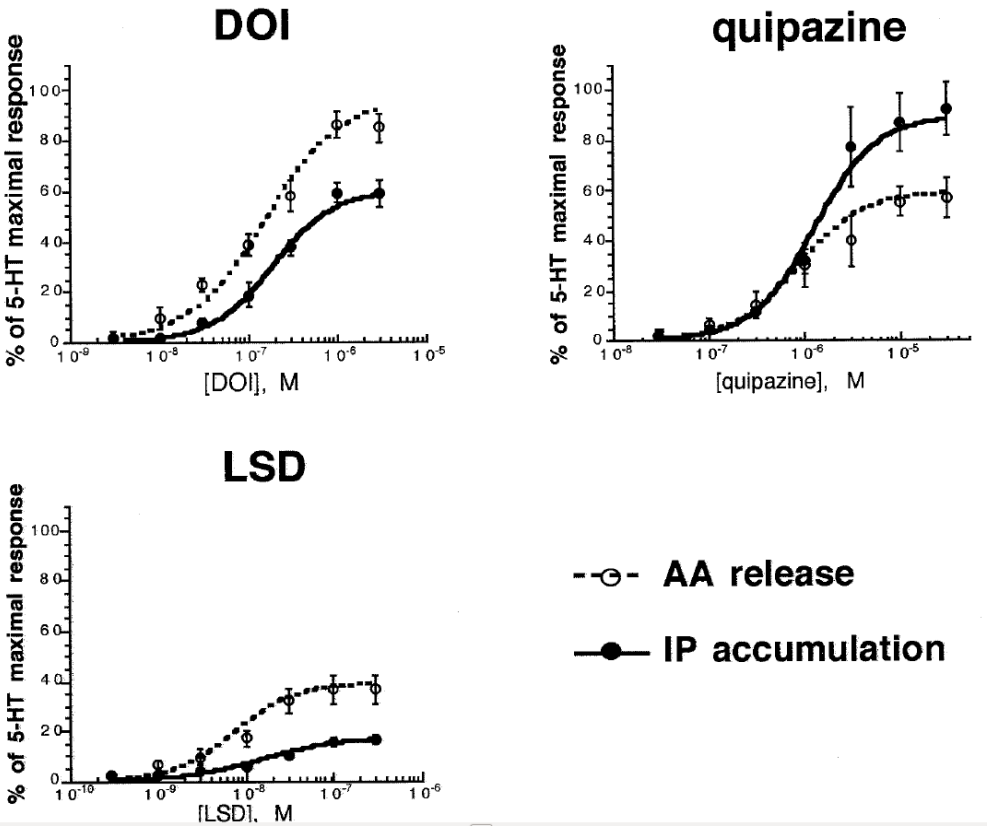
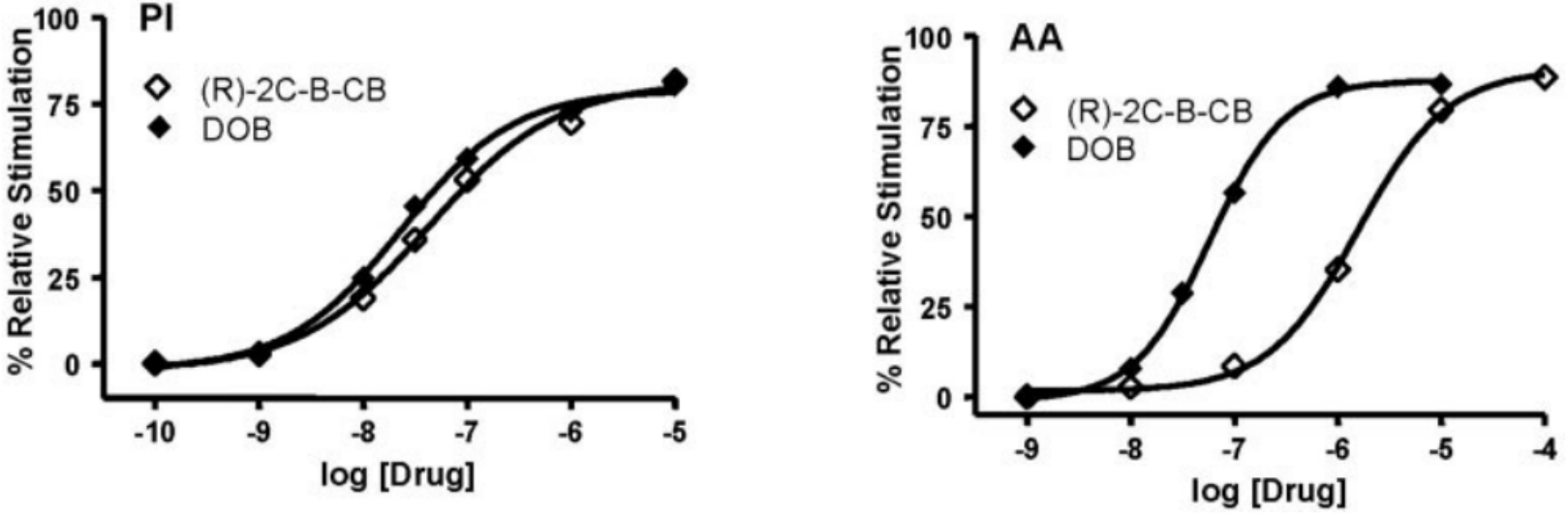


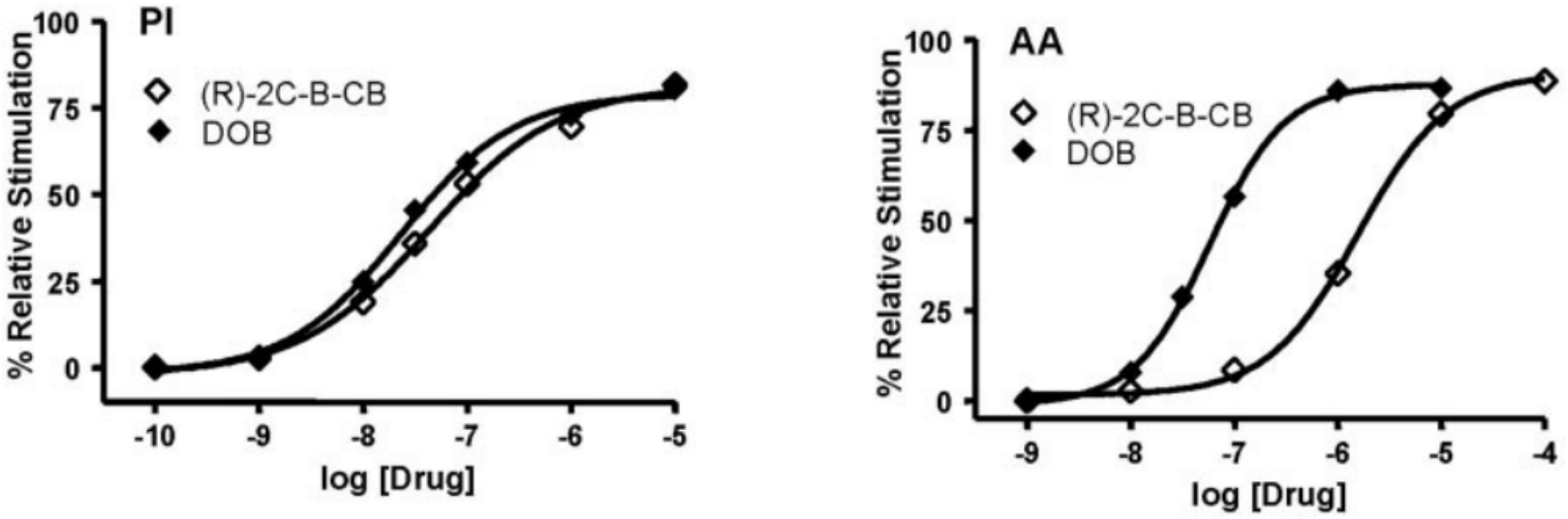
Serotonine receptor 5 – HT_{2A}



- $(R) - 2C - B - CB$ is biaised towards PI accumulation with respect to AA production, *compared to the reference agonist* DOB.

Urban et al., *J Pharmacol Exp Ther* (2007)

Serotonine receptor 5 – HT_{2A}



- $(R) - 2C - B - CB$ is biaised towards PI accumulation with respect to AA production, *compared to the reference agonist* DOB.

$\Rightarrow$ Bias due to an EC_{50} difference.

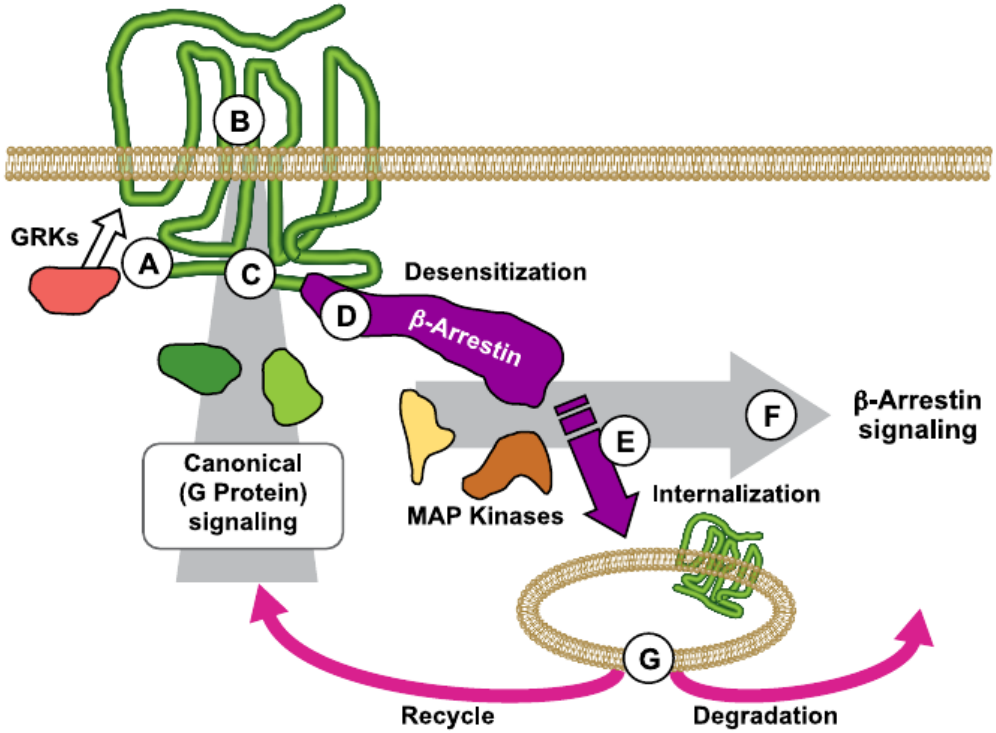
Urban et al., *J Pharmacol Exp Ther* (2007)

Many more examples on G Protein Coupled Receptor

Many GPCR's are known to have biased ligands

- G vs β-arrestin dependent signaling pathway

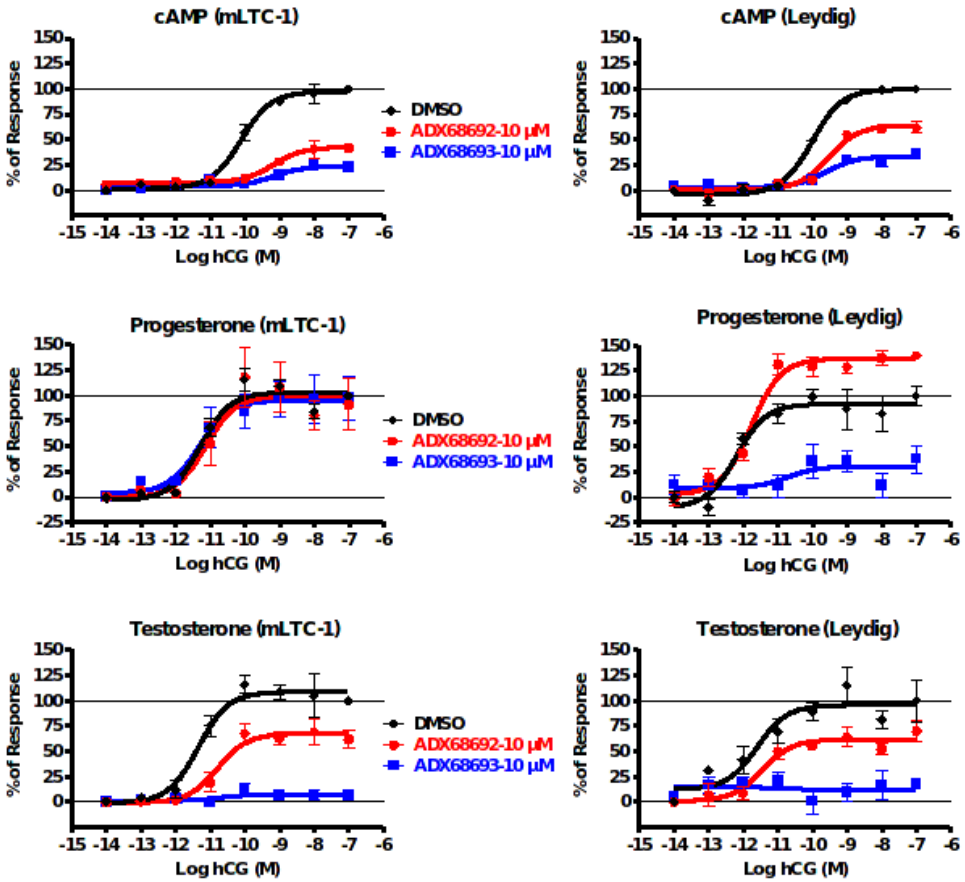
Kenakin, *Chem Rev* (2017)



Biased at more "integrated" response : Steroidogenesis modulated by NAM

Some negative allosteric modulators (NAM) can biased Progesterone production with respect to Testosterone production, under stimulation of LH/CG receptor by hCG.

Ayoub et al., *Mol. Cell. Endocrinol* (2016)



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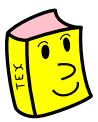
$\Rightarrow$ Selective (biased) allosteric modulation



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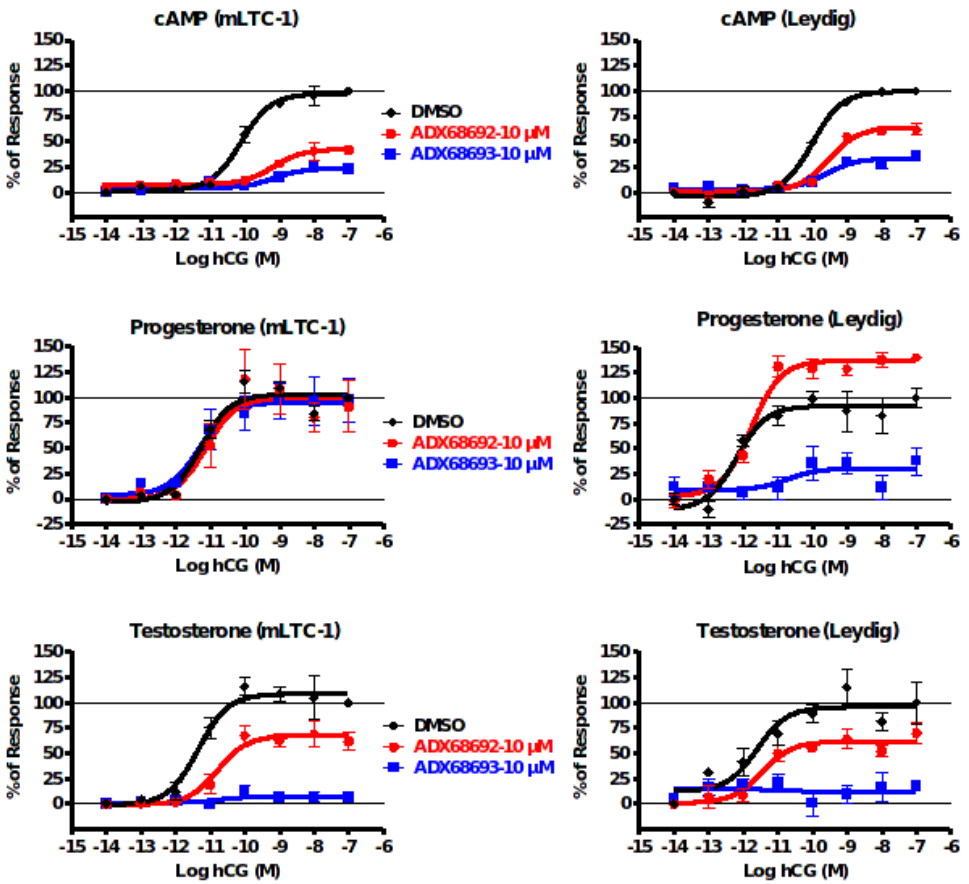


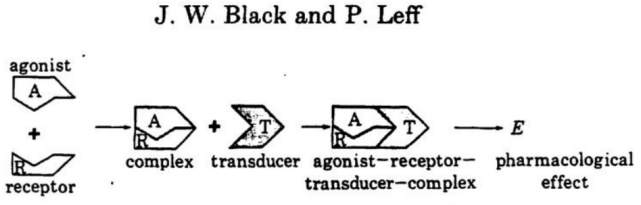
Outline

Operational model

Dose-response data are fitted with the function

$$y = E_{tot} \frac{\tau^n [L]^n}{([L] + Ka)^n + \tau^n [L]^n} .$$

- Response at equilibrium of a Michaelis-Menten type model.
- $Ka =$ **Dissociation constant** of the couple Ligand/Receptor
- $\tau =$ **Efficacy coefficient** of the transduction pathway



Black and Leff, *Proc. R. Soc. Lond. B* (1983)

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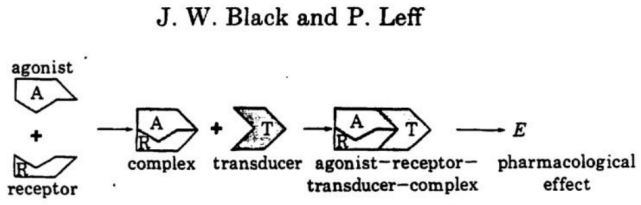
For $n = 1$,

- $EC_{50} = \frac{Ka}{\tau+1}$
- Efficacy $y_\infty / E_{tot} = \frac{\tau}{\tau+1}$



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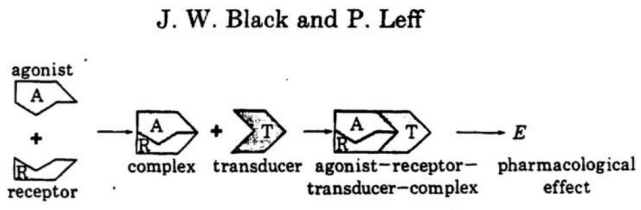
Then, we define

$\Rightarrow$ **Transduction coefficient** :

$$R := \log\left(\frac{\tau}{Ka}\right)$$



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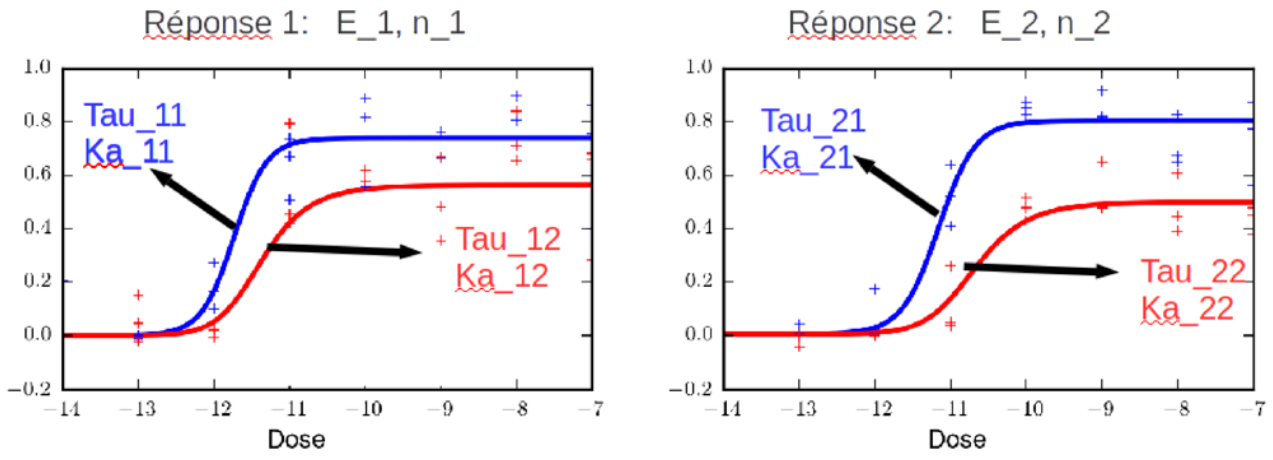
Bias quantification : with the operational model



Two ligands ($j = 1, 2$) and **two** measured responses ($i = 1, 2$) :
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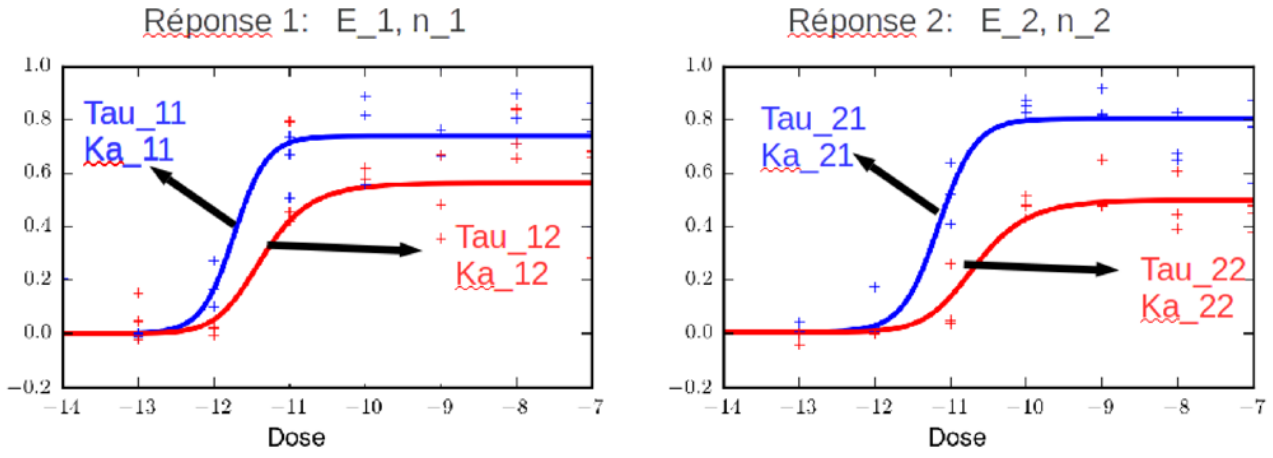

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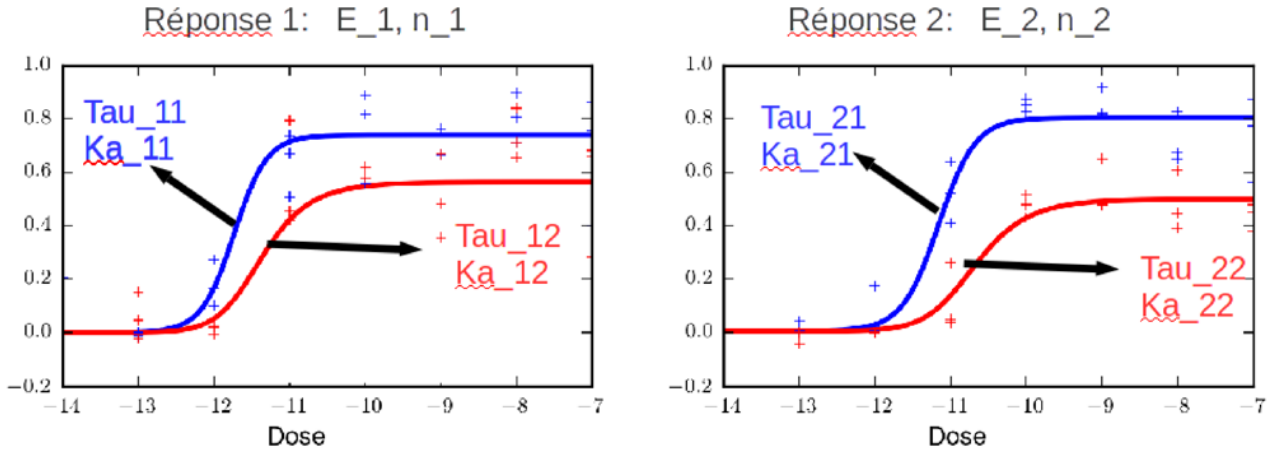

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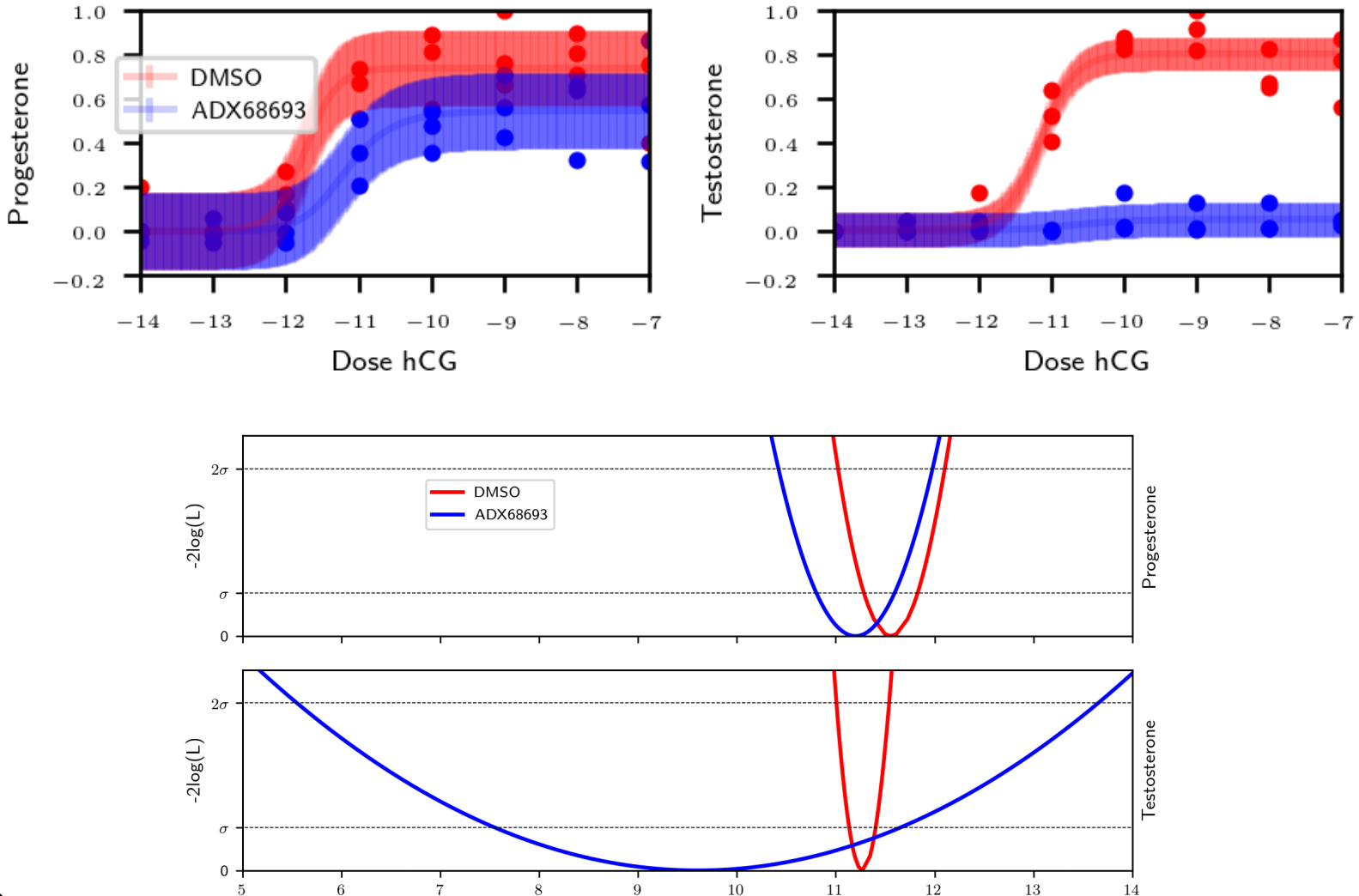
The **Bias** is then defined by

$$\Delta\Delta \log(\tau/Ka) = \Delta_2 \log(\tau/Ka) - \Delta_1 \log(\tau/Ka)$$

Statistical consideration : parameter confidence interval and (un-)identifiability



Data2Dynamics : Raue A., et al. *Bioinformatics* (2015)

Outline

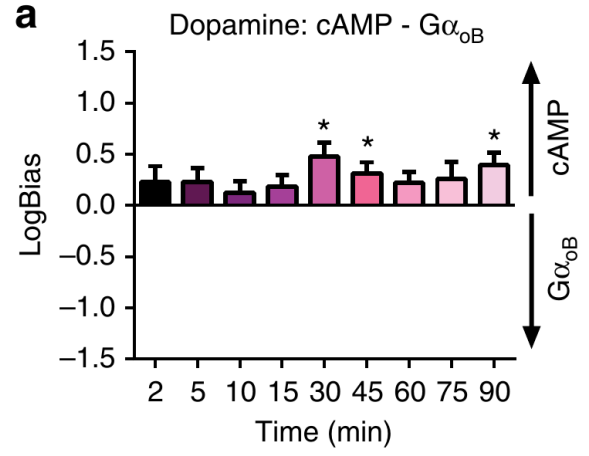
Time-dependent bias ?

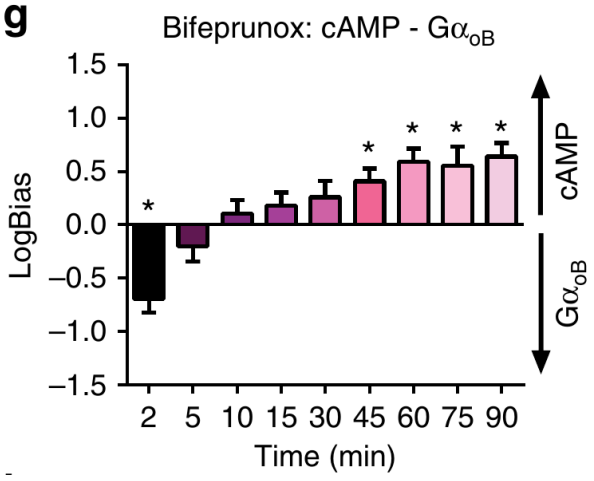
The role of kinetic context in apparent biased agonism at GPCRs

Carmen Klein Herenbrink[1], David A. Sykes[2], Prashant Donthamsetti[3,4], Meritxell Canals[1], Thomas Coudrat[1], Jeremy Shonberg[5], Peter J. Scammells[5], Ben Capuano[5], Patrick M. Sexton[1], Steven J. Charlton[2], Jonathan A. Javitch[3,4,6], Arthur Christopoulos[1] & J Robert Lane[1]

- Bias value may change according to the response time after stimulation.
- Kinetic explanation : Ligands with a slow binding kinetics may have changing bias value according to time.

Klein Herenbrink et al., *Nat. Commun* (2016)





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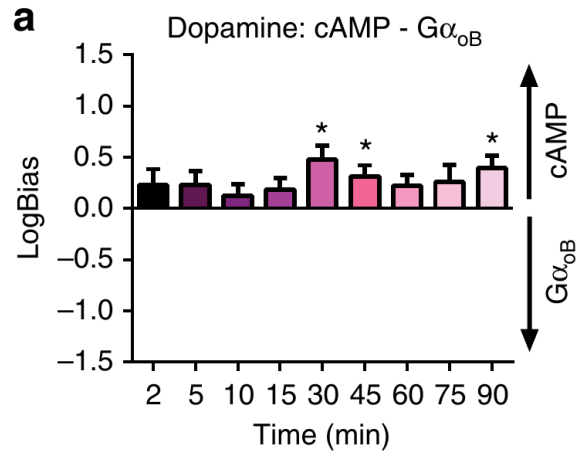
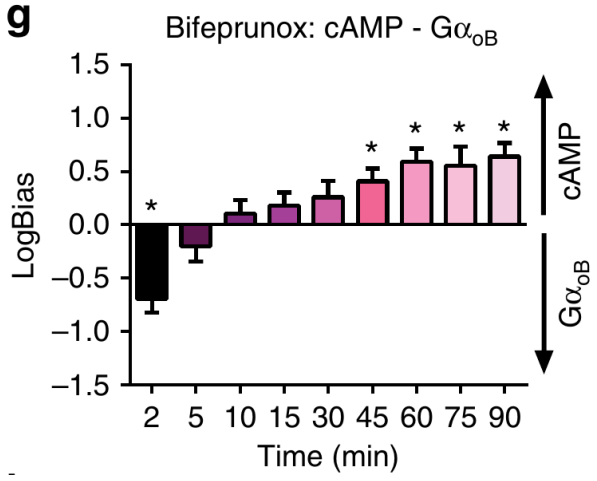
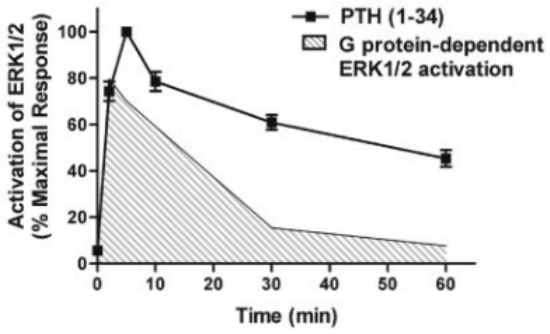
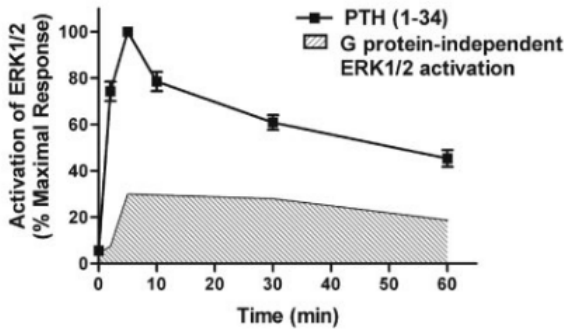
⇒ **We need to take into account dynamic patterns in bias quantification**





Physiological role of a kinetic profile (PTHR)

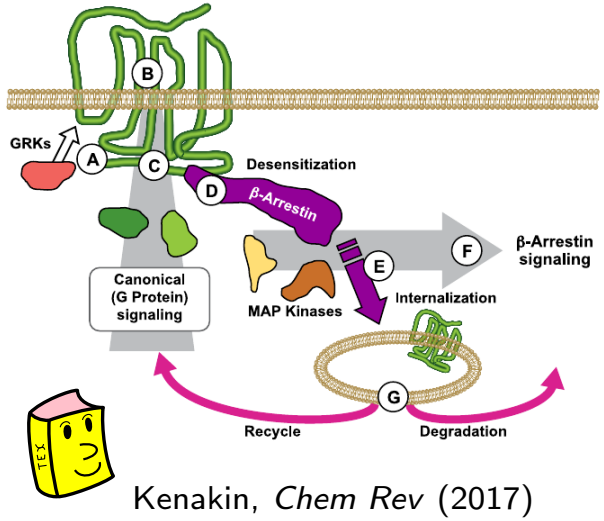




Gesty-Palmer et al., *J. Biol. Chem* (2006)

The mitogen-activated protein kinase ERK activation profile is the sum of two different activation pathways (G vs β-arrestin dependent), linked to two different spatial distribution of activated ERK (nucleus vs cytoplasm).

The balance of activation of the two pathways controls cell fate outcome.



Kenakin, *Chem Rev* (2017)

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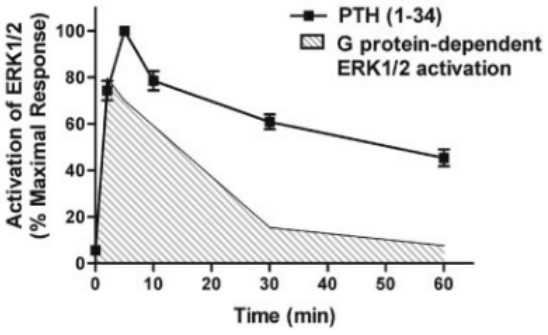

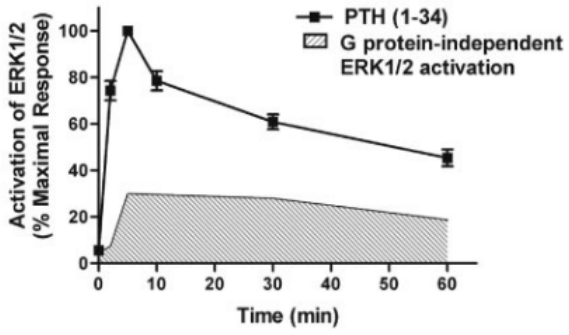

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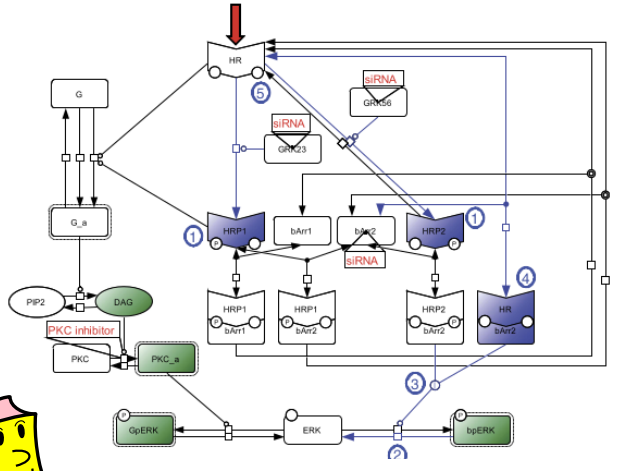
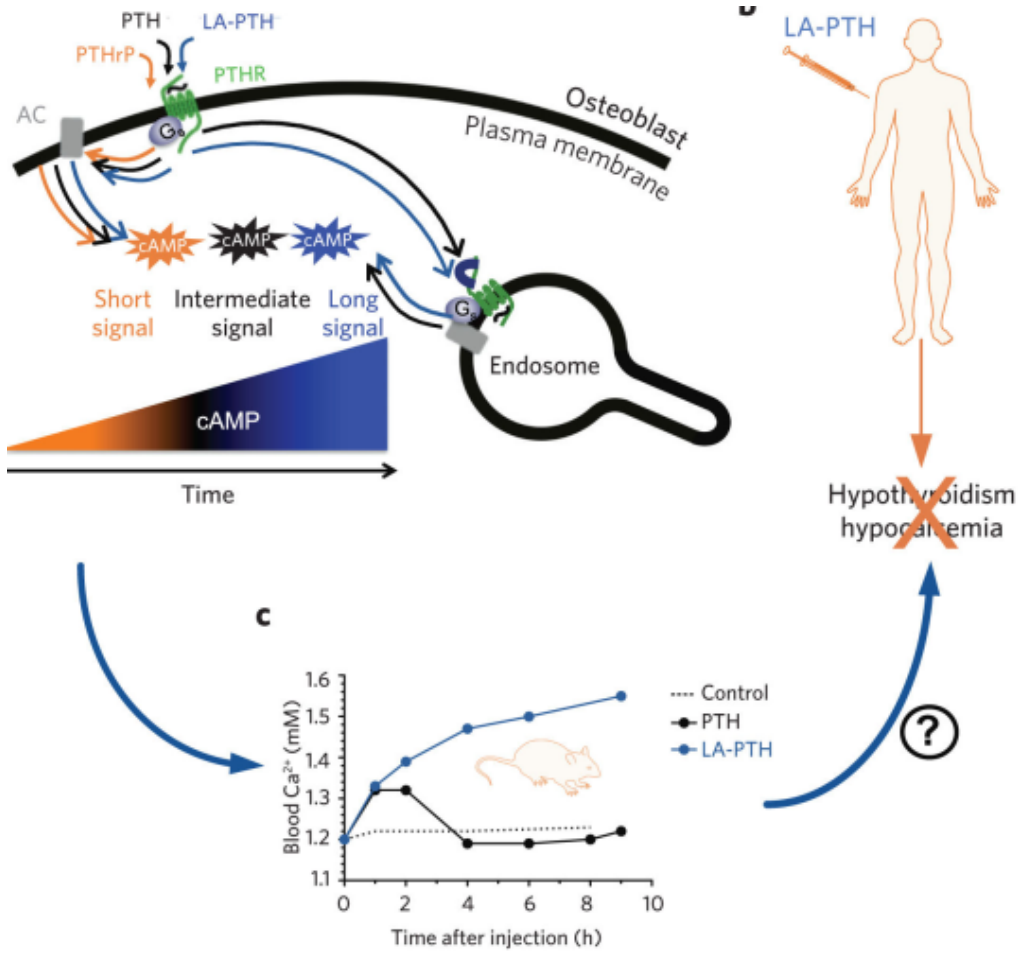
The balance of activation of the two pathways is finely controlled by G protein coupled receptors kinases.



Heitzler et al., *Mol. Sys. Biol.* (2012)

Physiological role of a kinetic profile (PTHR)

Short and Long acting Ligands activates the same secondary effector molecules (cAMP) trough different mechanisms, and lead to different physiological responses.

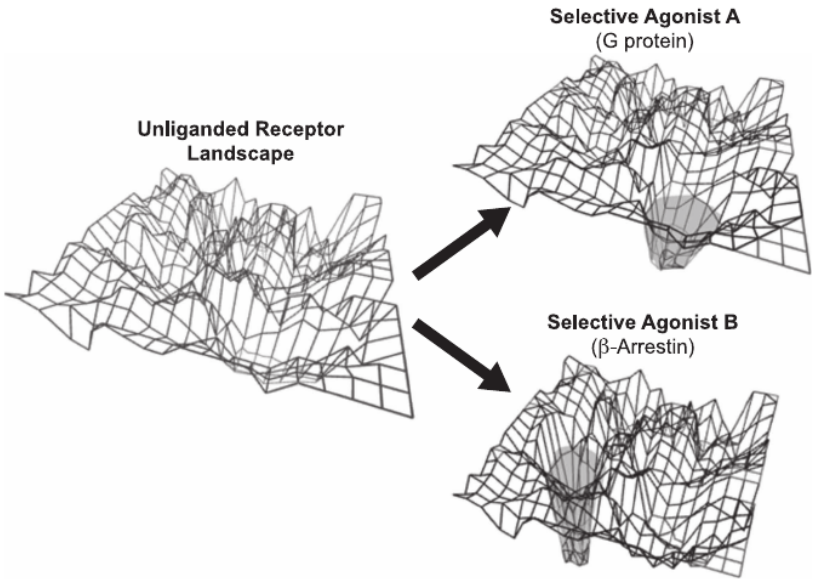


Vilardaga et al., *Nat Chem Biol* (2014)

Outline

How to quantify dynamic bias ?

- Ligand-specific modification of the energetic landscape controls signaling bias

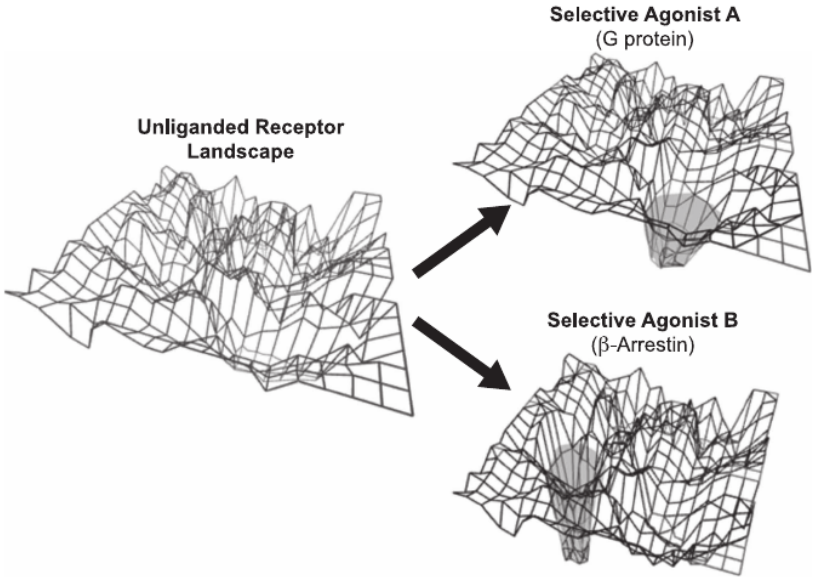


Kenakin, *J Pharmacol Exp Ther* (2011)

How to quantify dynamic bias ?

- Ligand-specific modification of the energetic landscape controls signaling bias

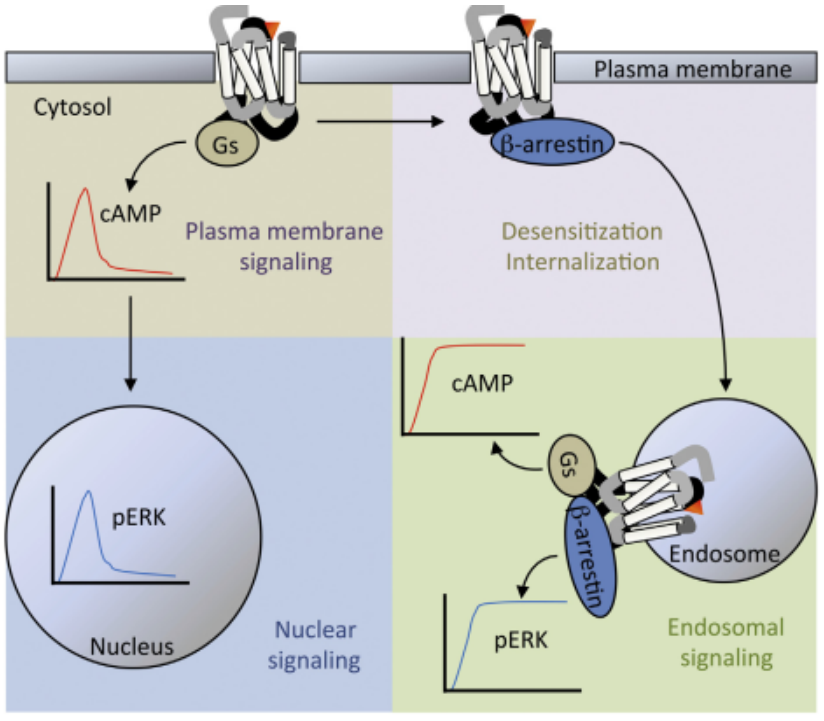
→ However this information is barely accessible.



Kenakin, *J Pharmacol Exp Ther* (2011)

How to quantify dynamic bias ?

- Ligand-specific modification of the energetic landscape controls signaling bias
- But we have access to the kinetic profile of receptor downstream signaling

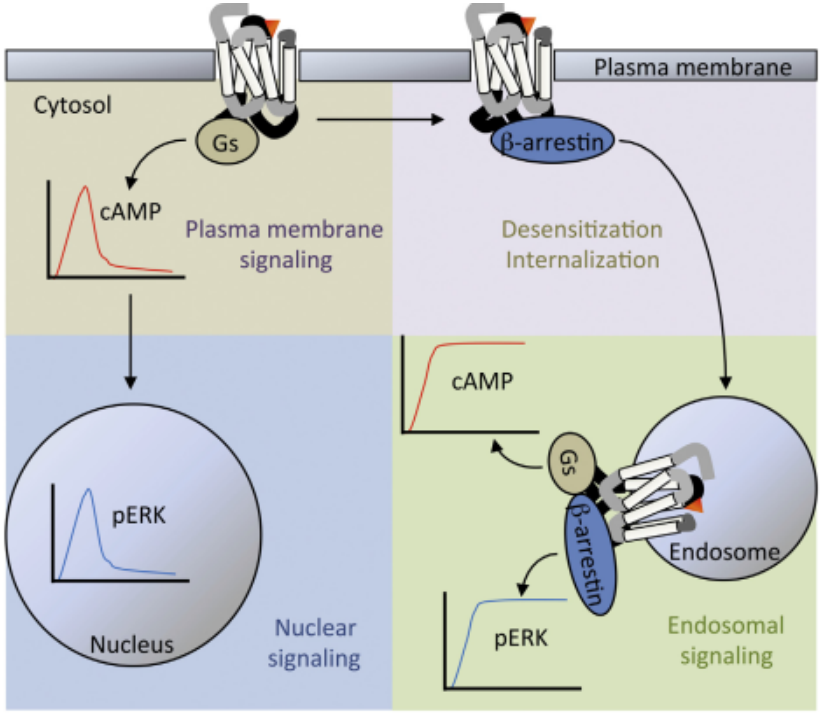


Poupon and Reiter, *Cell. Endocrin. in Health and Disease.* (2014)

How to quantify dynamic bias ?

- Ligand-specific modification of the energetic landscape controls signaling bias
- But we have access to the kinetic profile of receptor downstream signaling

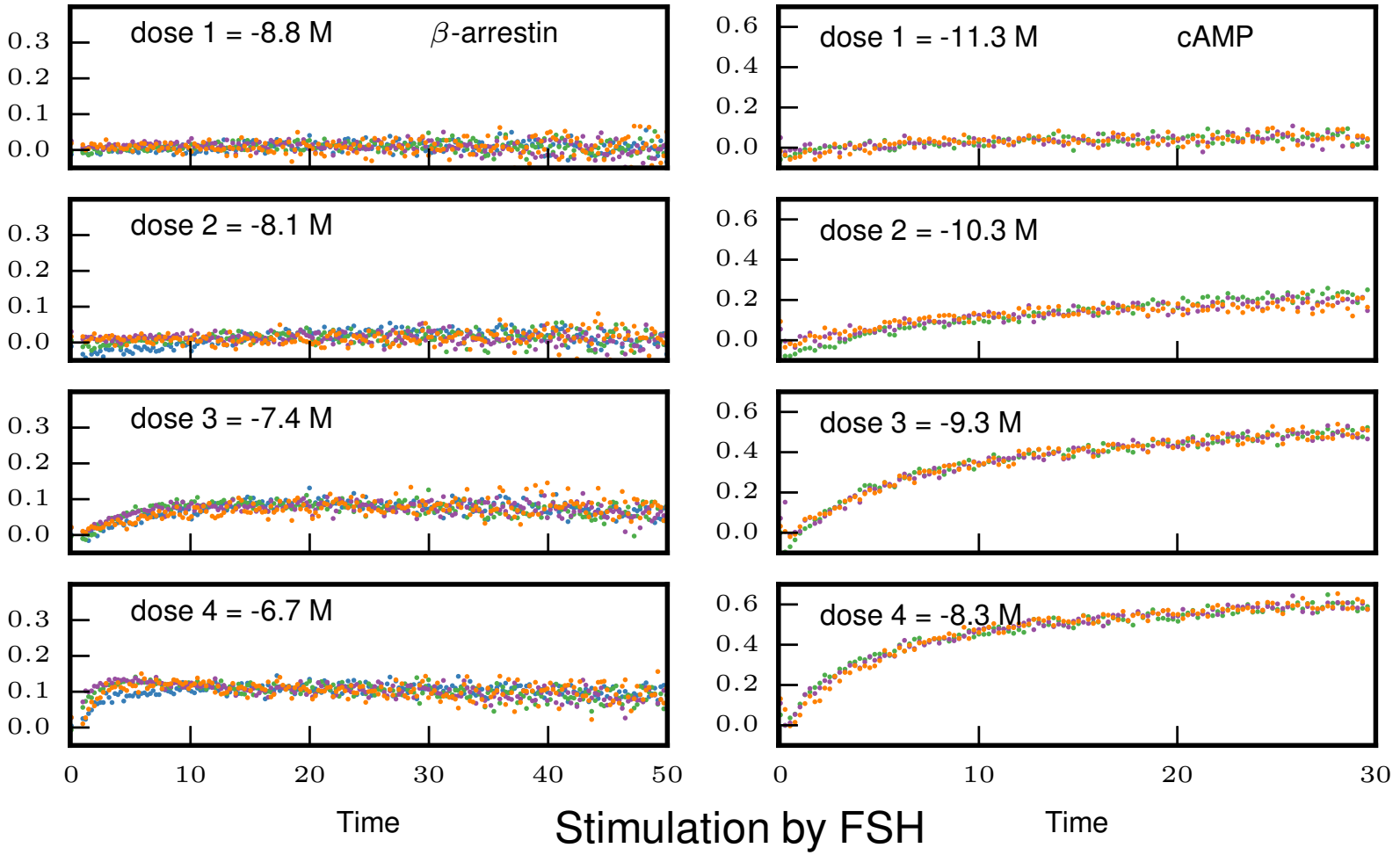
→ Dynamic modeling.



Poupon and Reiter, *Cell. Endocrin. in Health and Disease.* (2014)

Dynamic data (on FHSR in HEK cells)

Instead of focusing on dose-response curves, we deal with **kinetic data** performed at several doses (here : induced BRET data)



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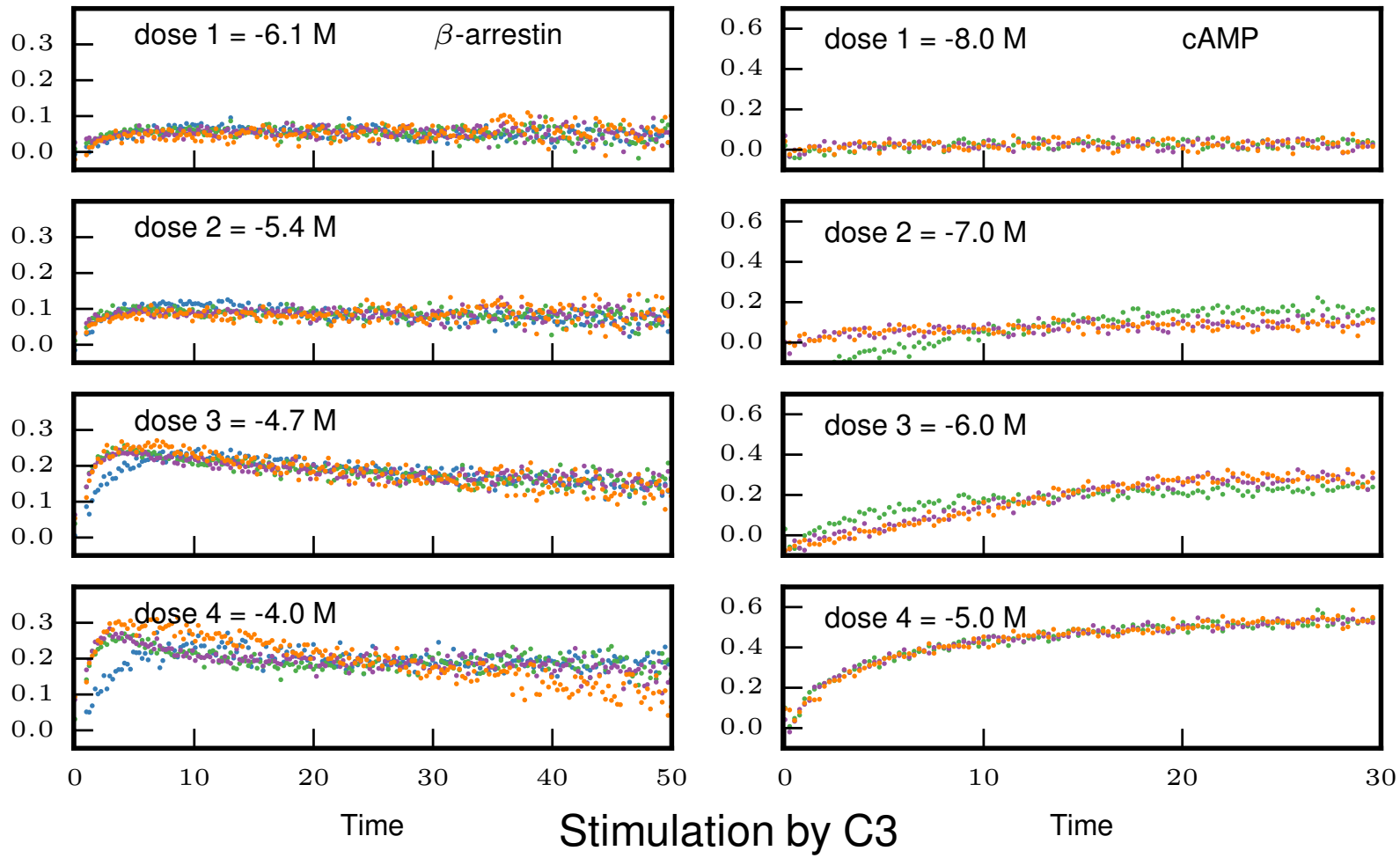
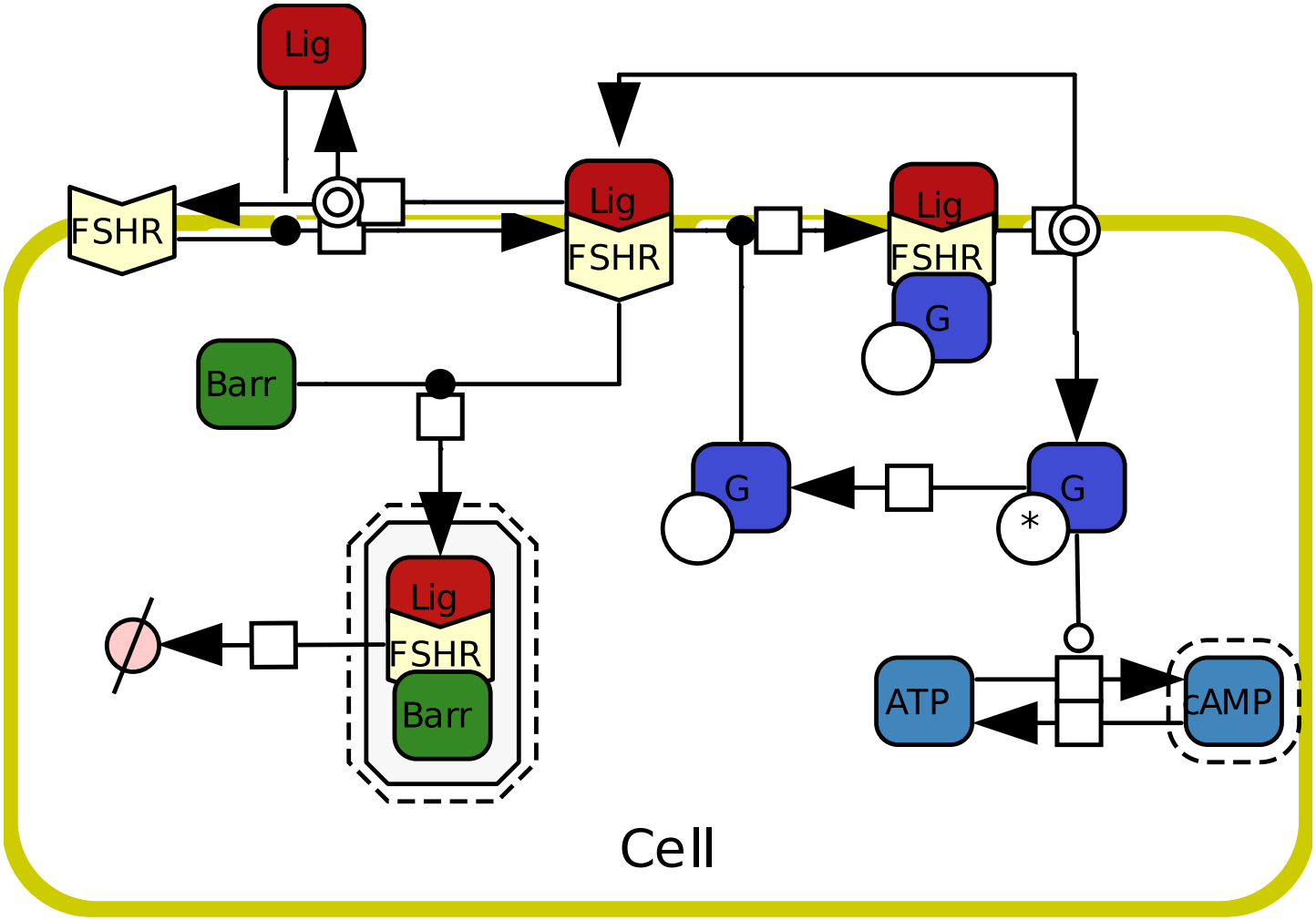


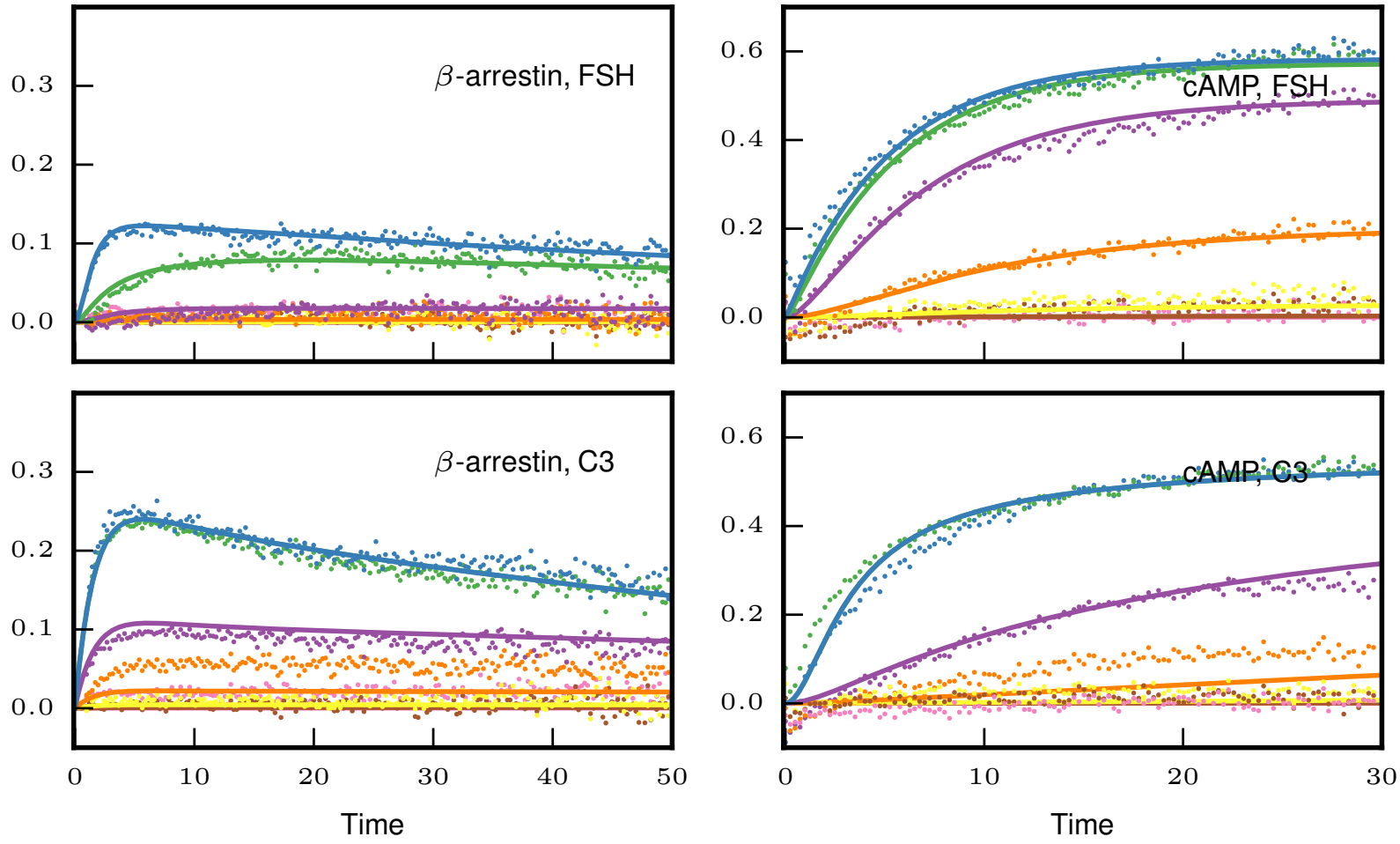
Principle of the methodology

I) We use chemical reaction network and ODE modeling (based on mass action law) to generate time series



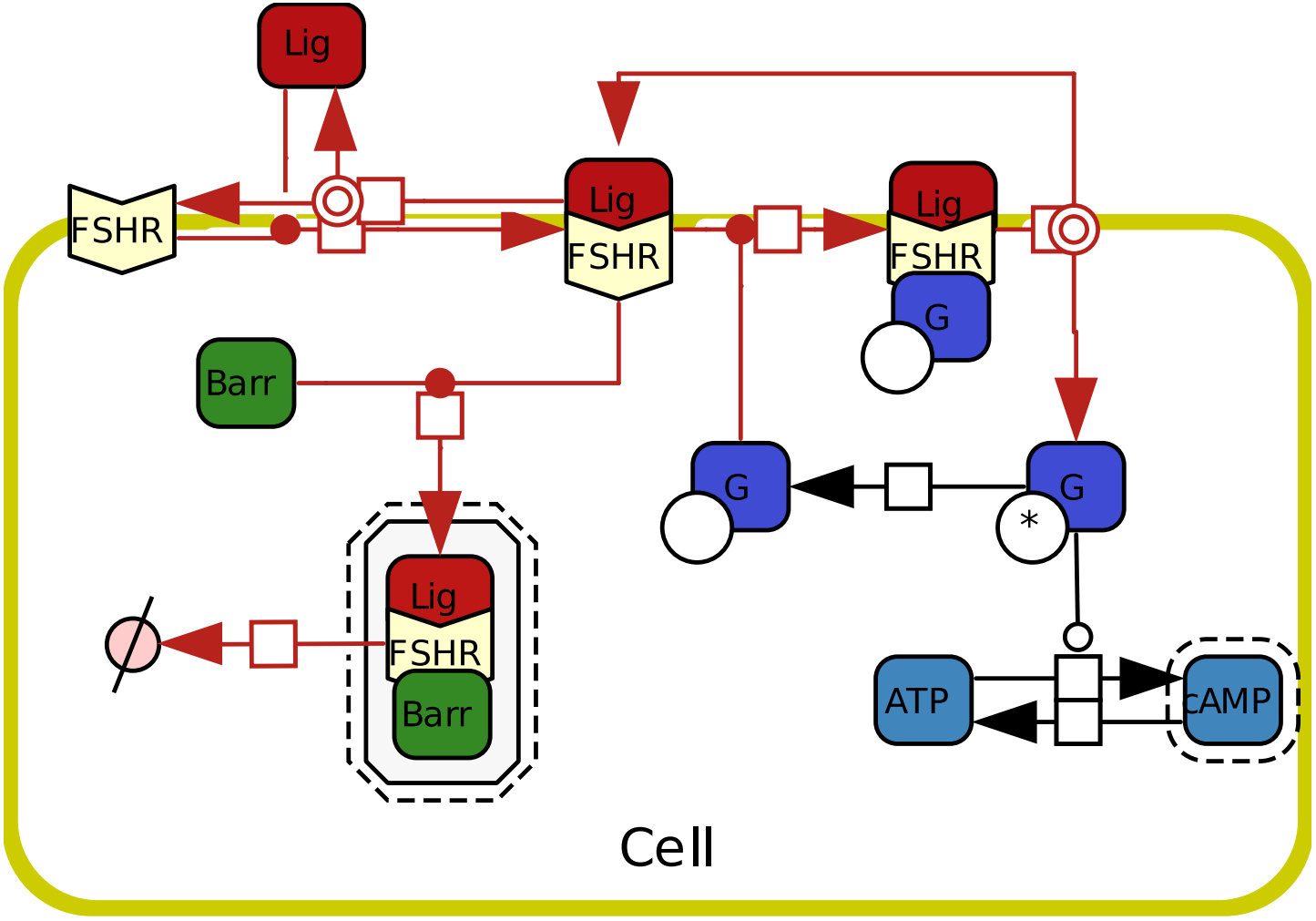
Principle of the methodology

I) We verify the network is able to accurately fit the data (one **separate fit** for each Ligand)



Principle of the methodology

II) We fit **all data at once**, using some **common** parameters (initial concentration of molecules, measurement parameters...) and some **different** ones (kinetic parameters...)



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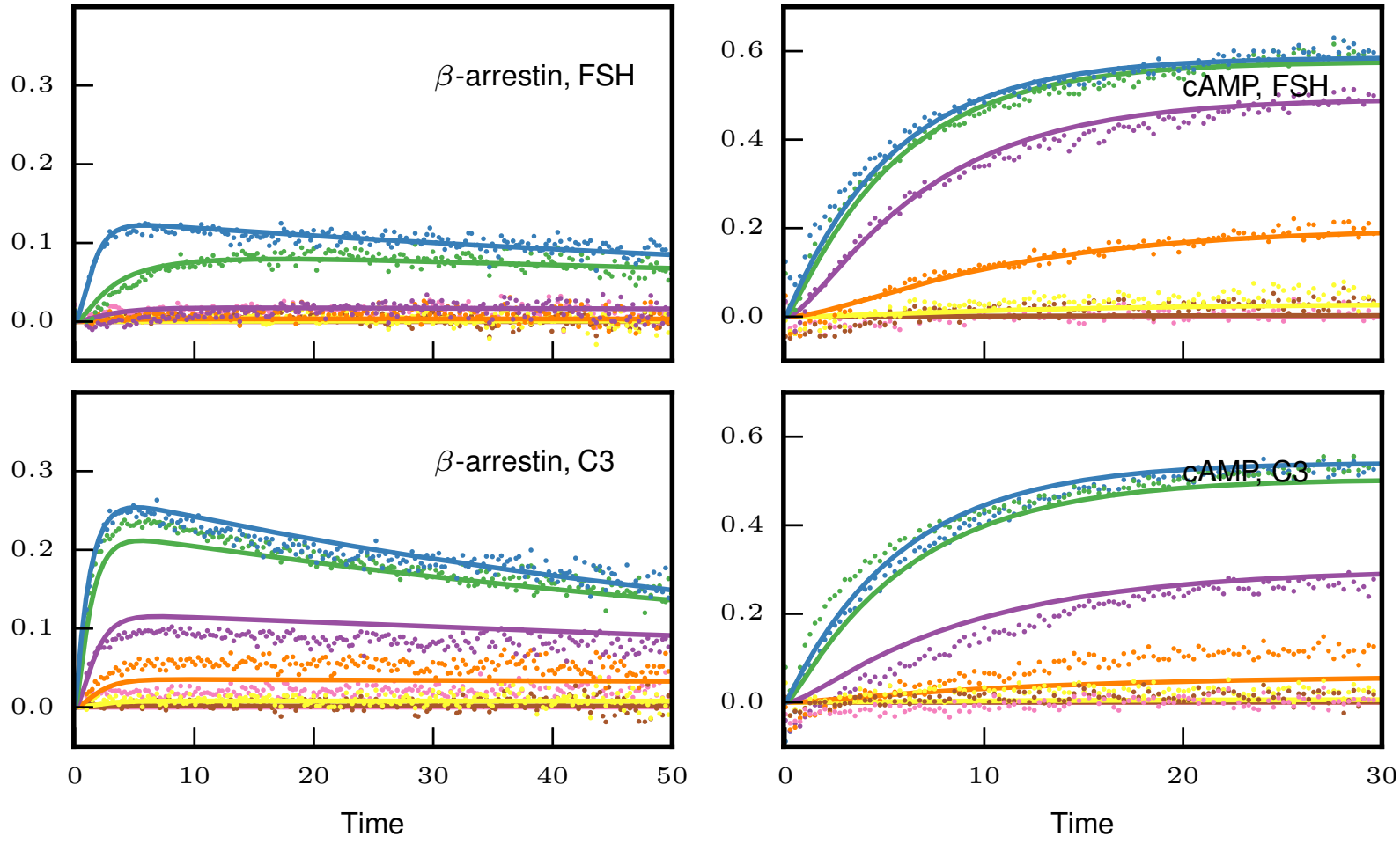
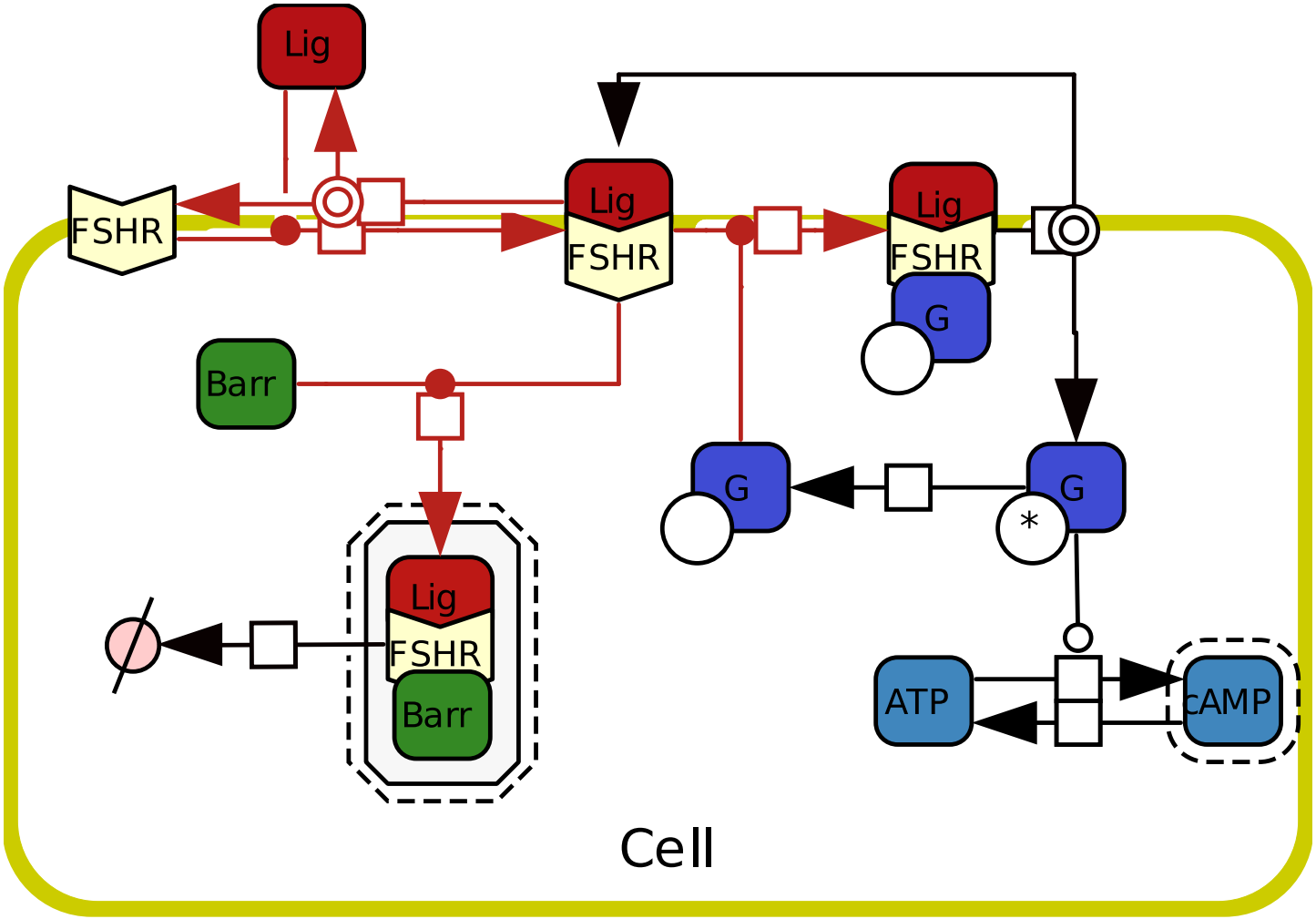


Principle of the methodology

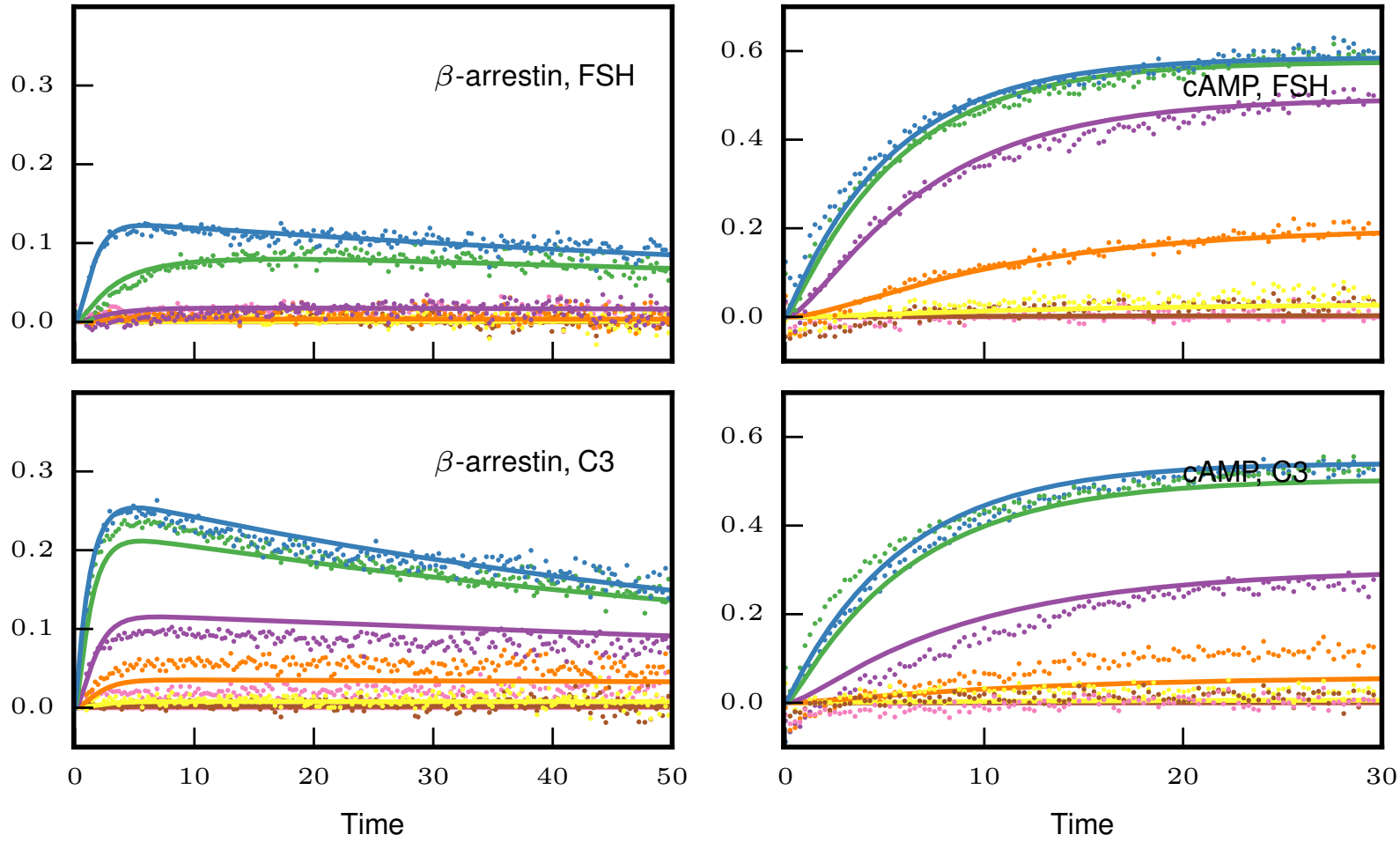
III) We use L^1-penalization to find **ligand specific parameters**

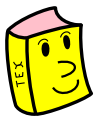


Data2Dyanmics : Steiert, Timmer and Kreutz, *Bioinformatics* (2016)

Principle of the methodology

III) We use L^1-penalization to find **ligand specific parameters**, keeping the fit 'as good as before'

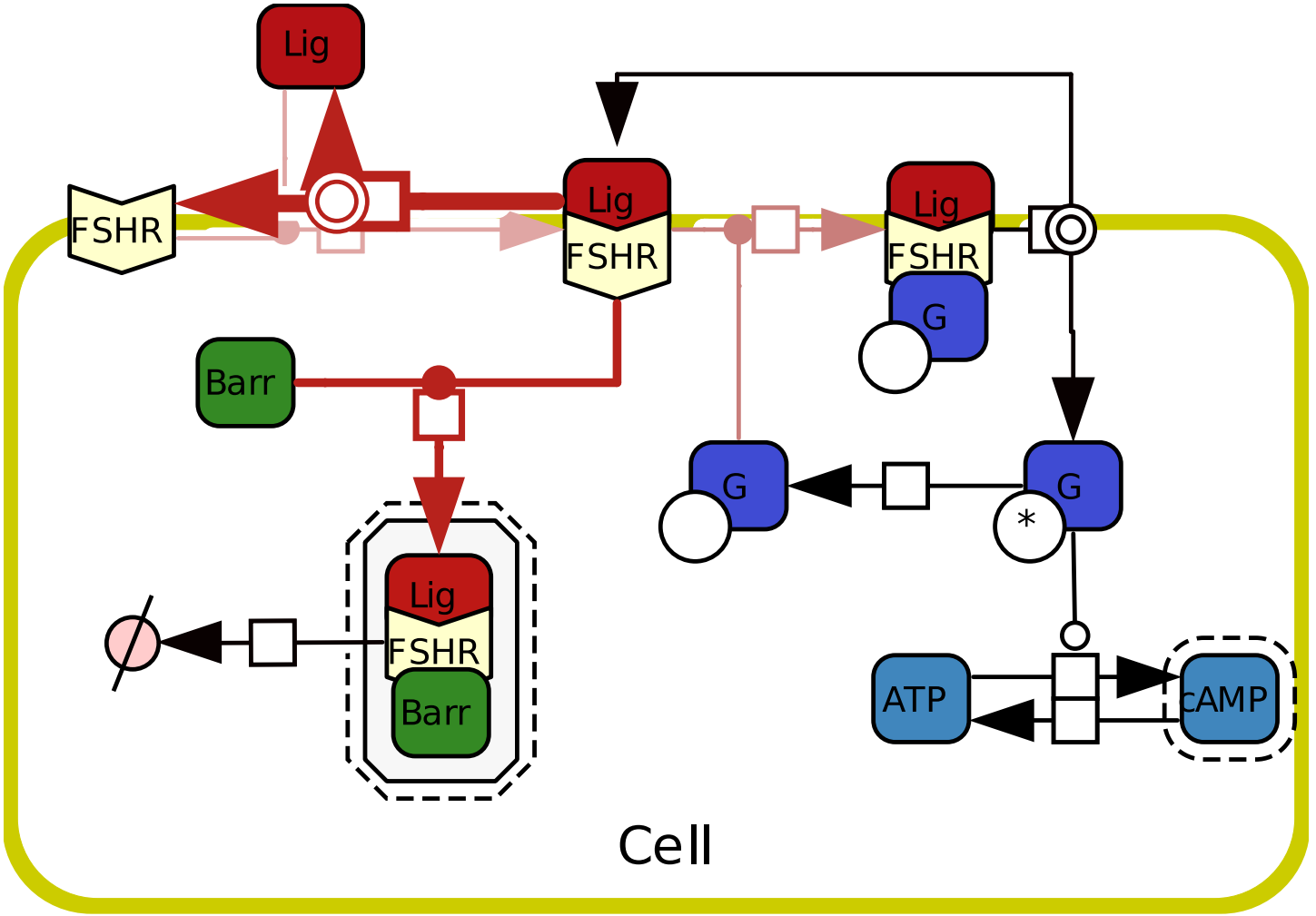




Steiert, Timmer and Kreutz, *Bioinformatics* (2016)

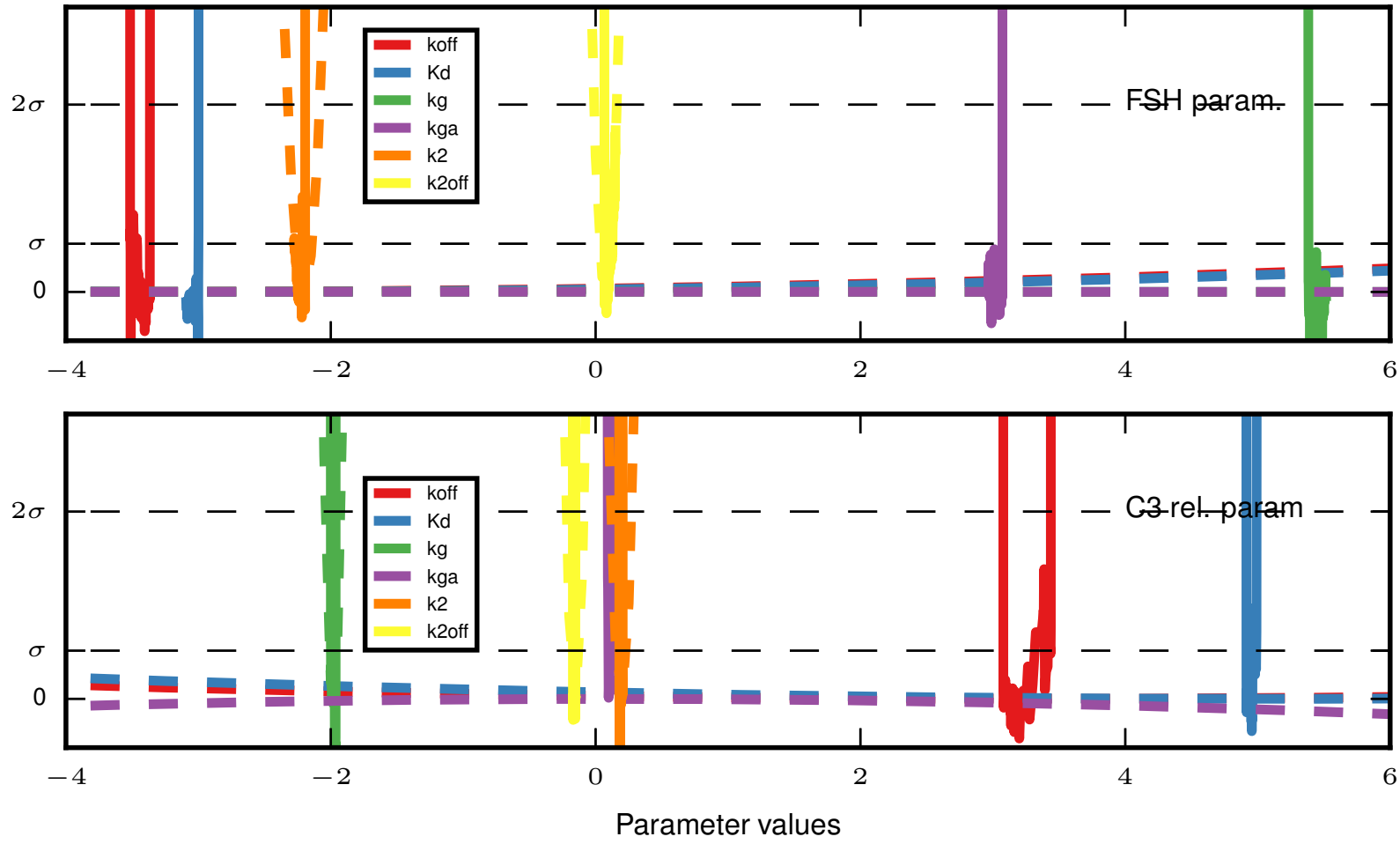
Principle of the methodology

IV) After re-optimization, the set of distinct (ligand-specific) kinetic parameters gives us an accurate description of ligand specificity.



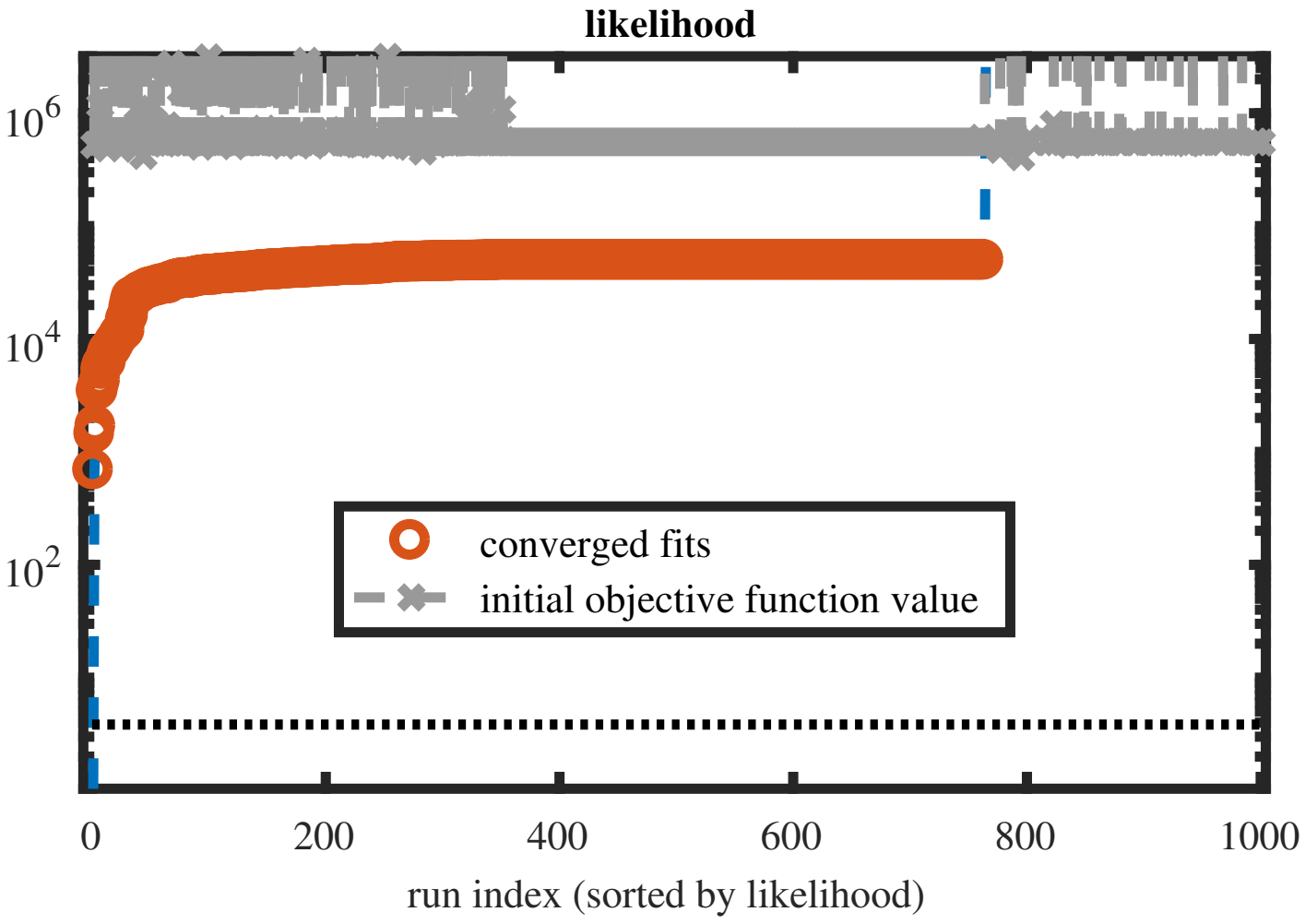
Principle of the methodology

V) Significant differences between parameters is assessed by statistical methods (Profile Likelihood)

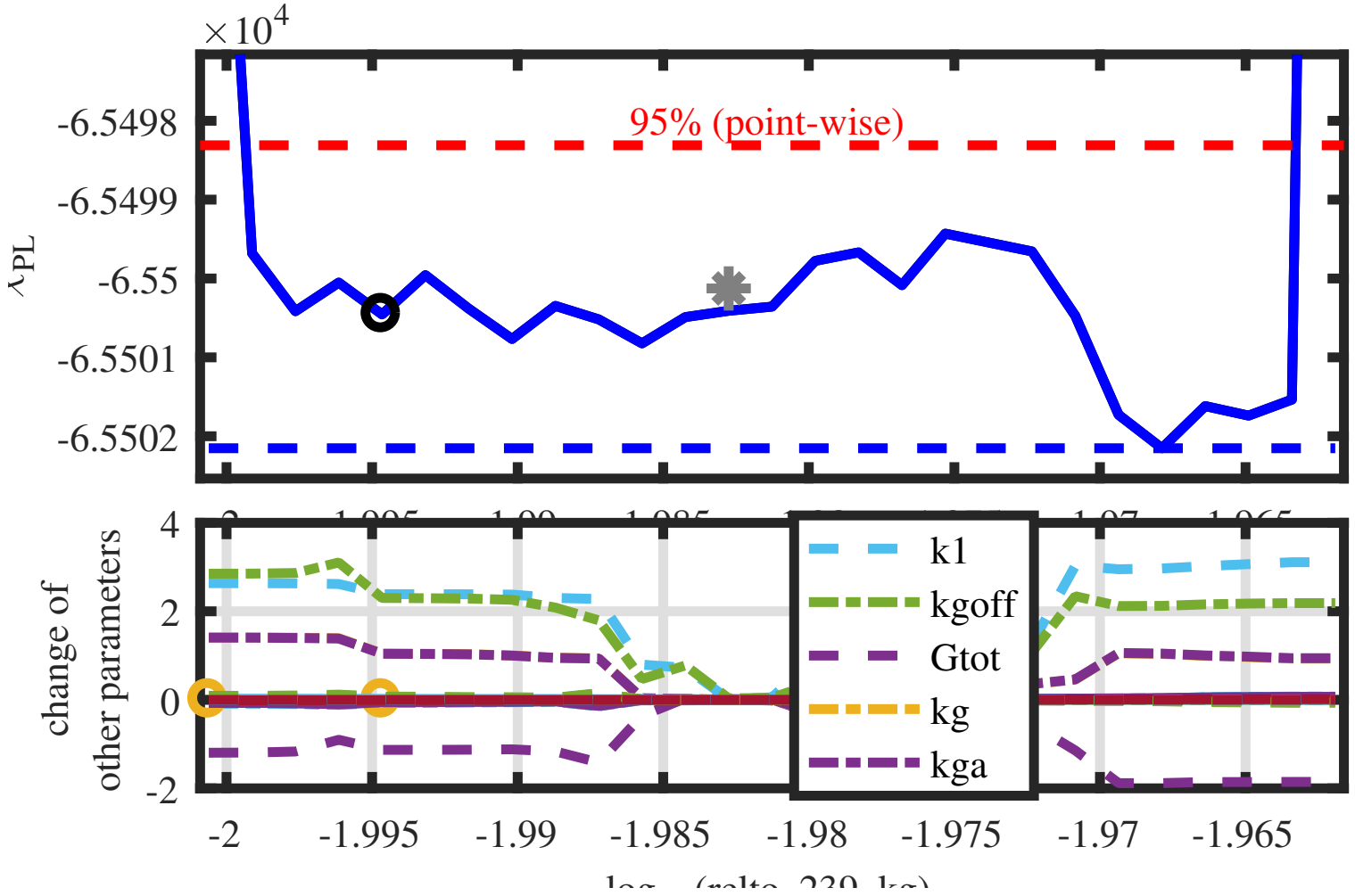


→**here :** C3 is biased towards β-arr, compared to cAMP, in comparison to FSH

Practical problems...

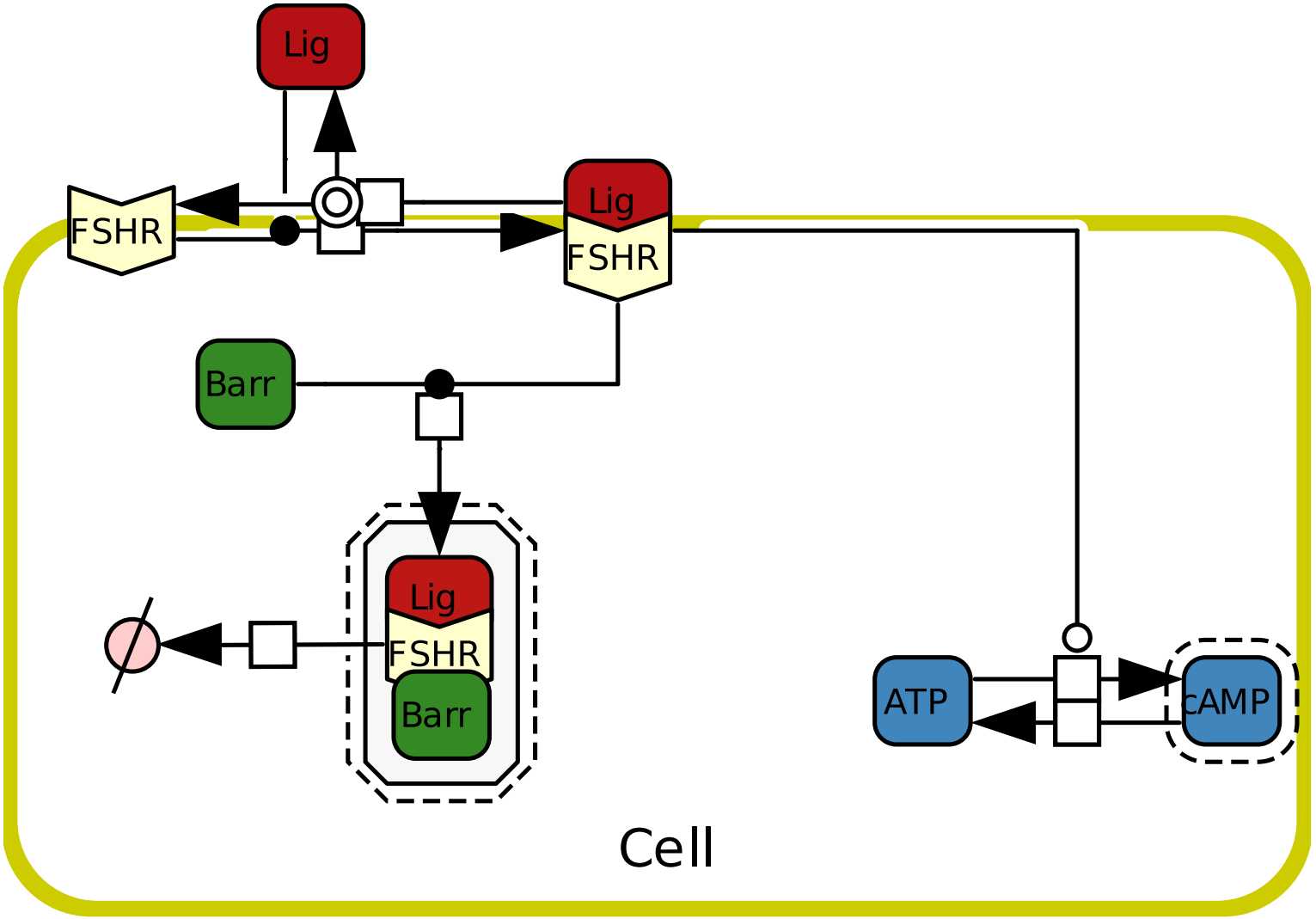


Practical problems...



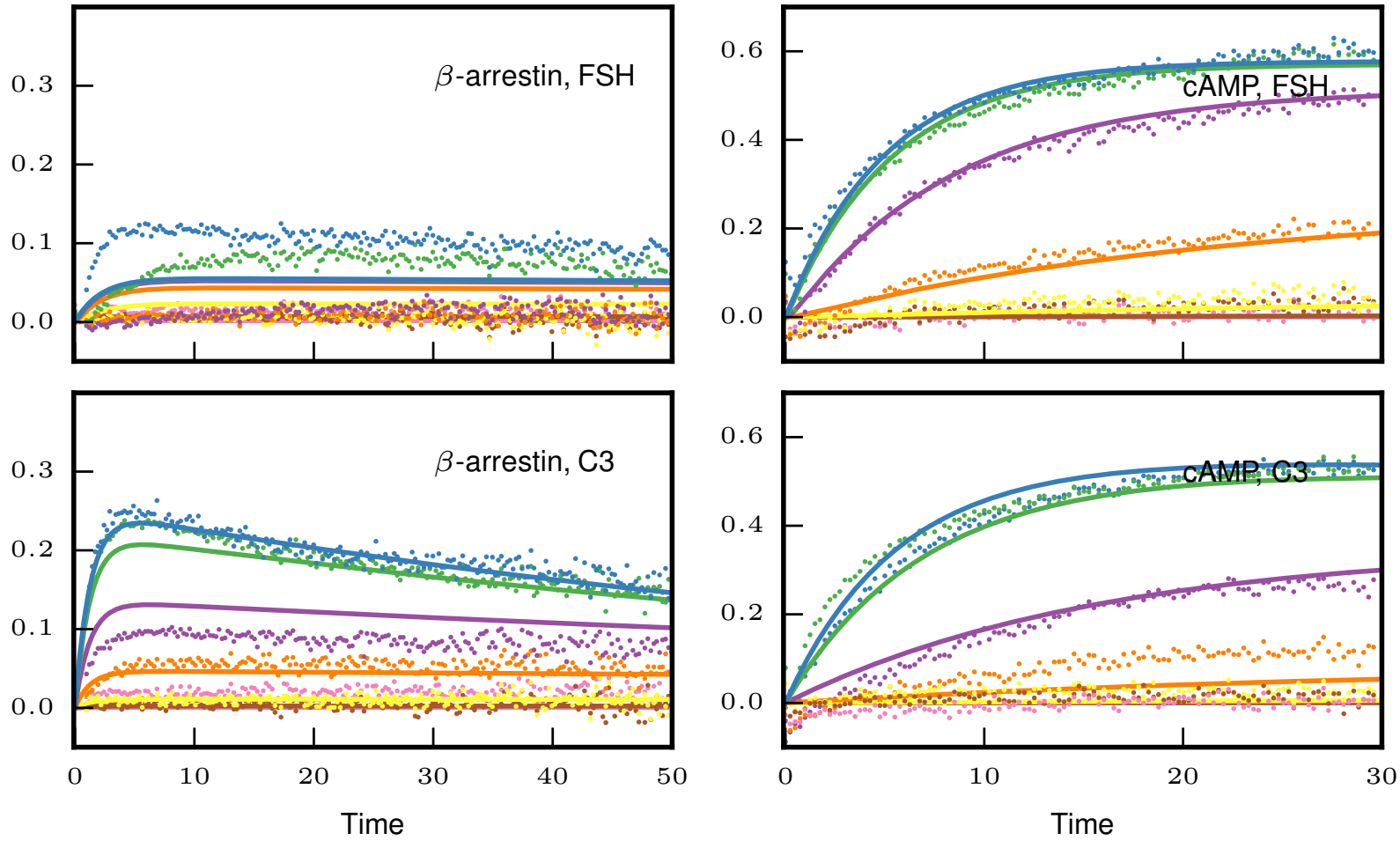
With a "simpler" model

Kinetic model without G-protein



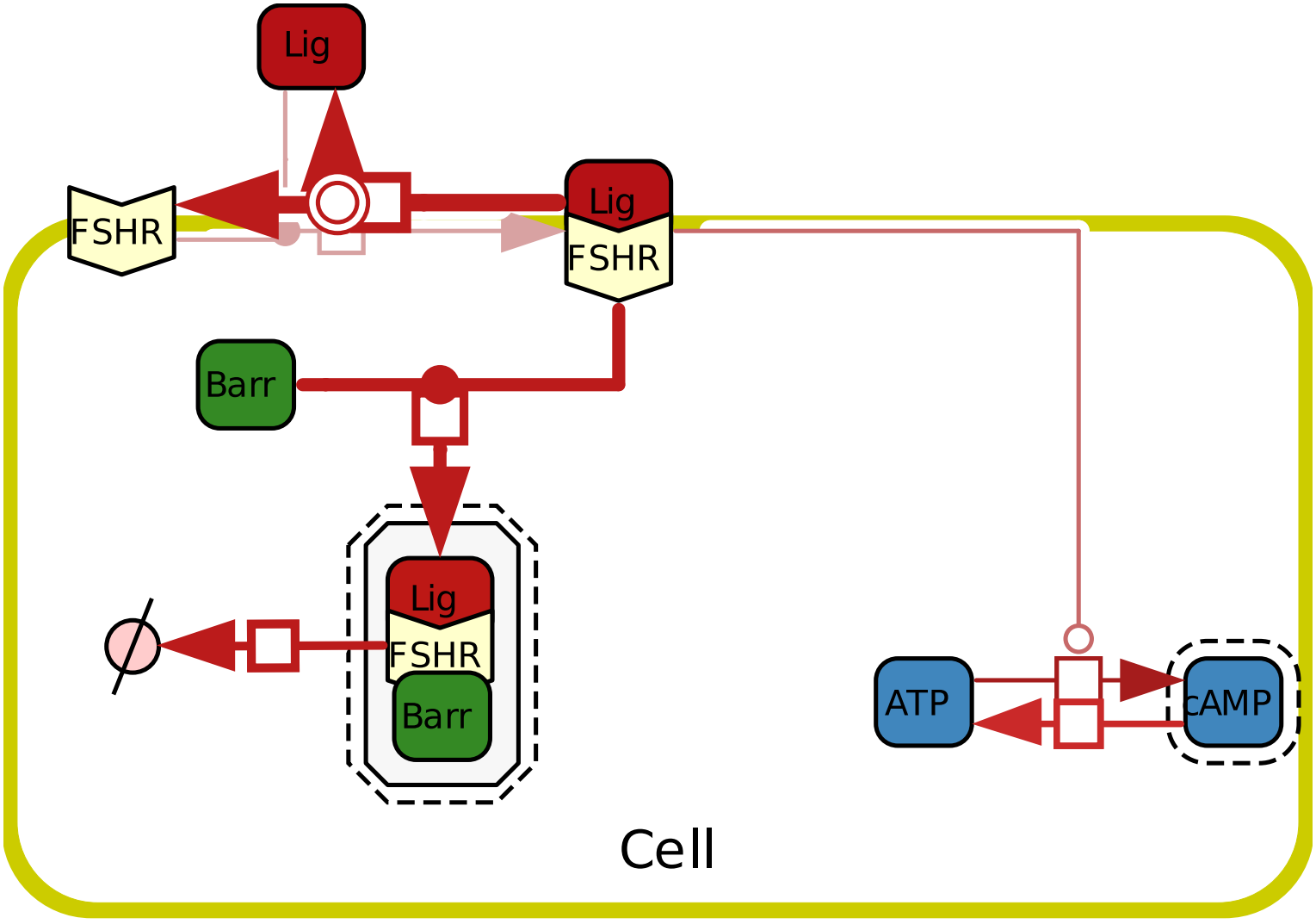
With a "simpler" model

We obtain a slightly worse fit



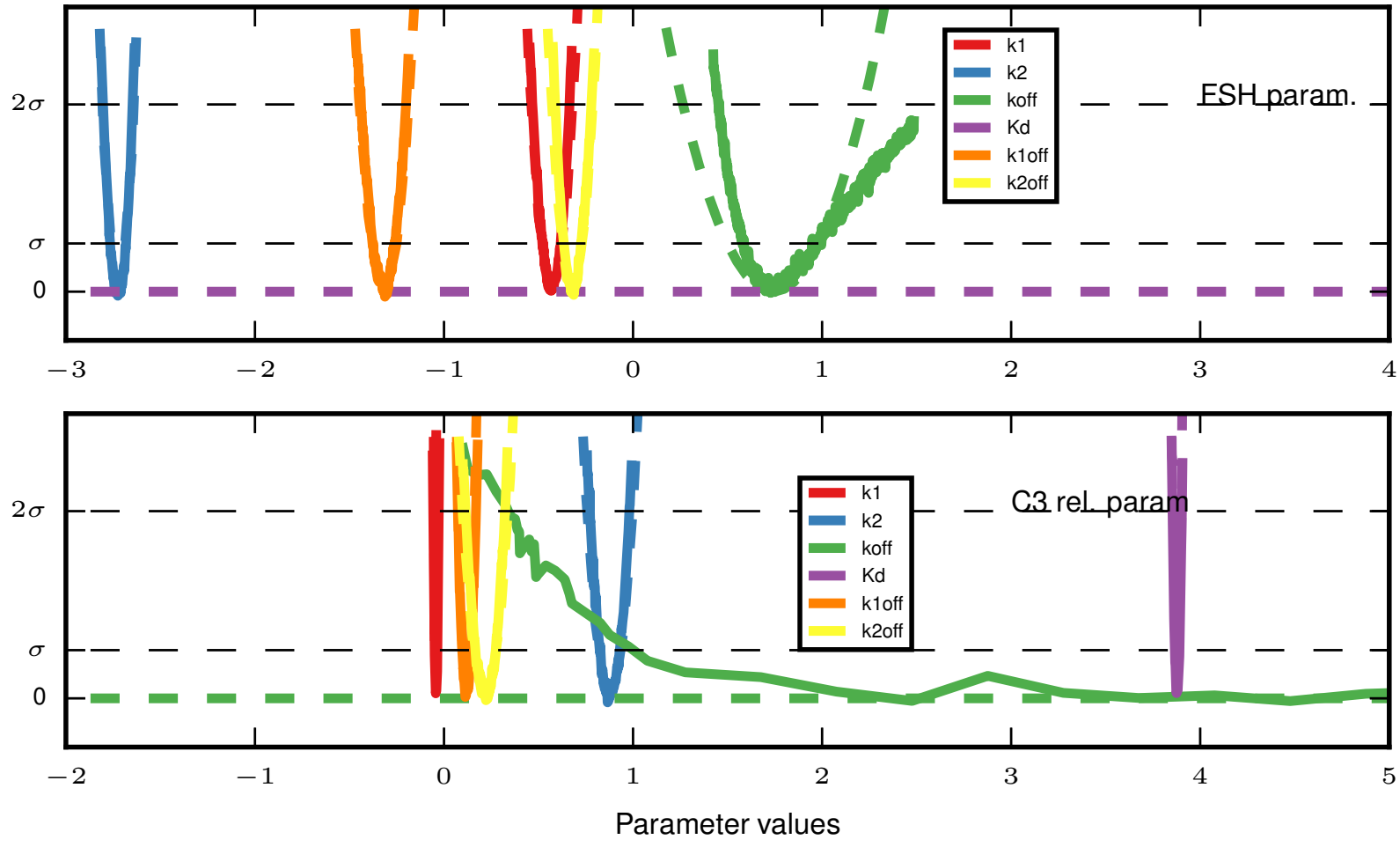
With a "simpler" model

But consistent results



With a "simpler" model

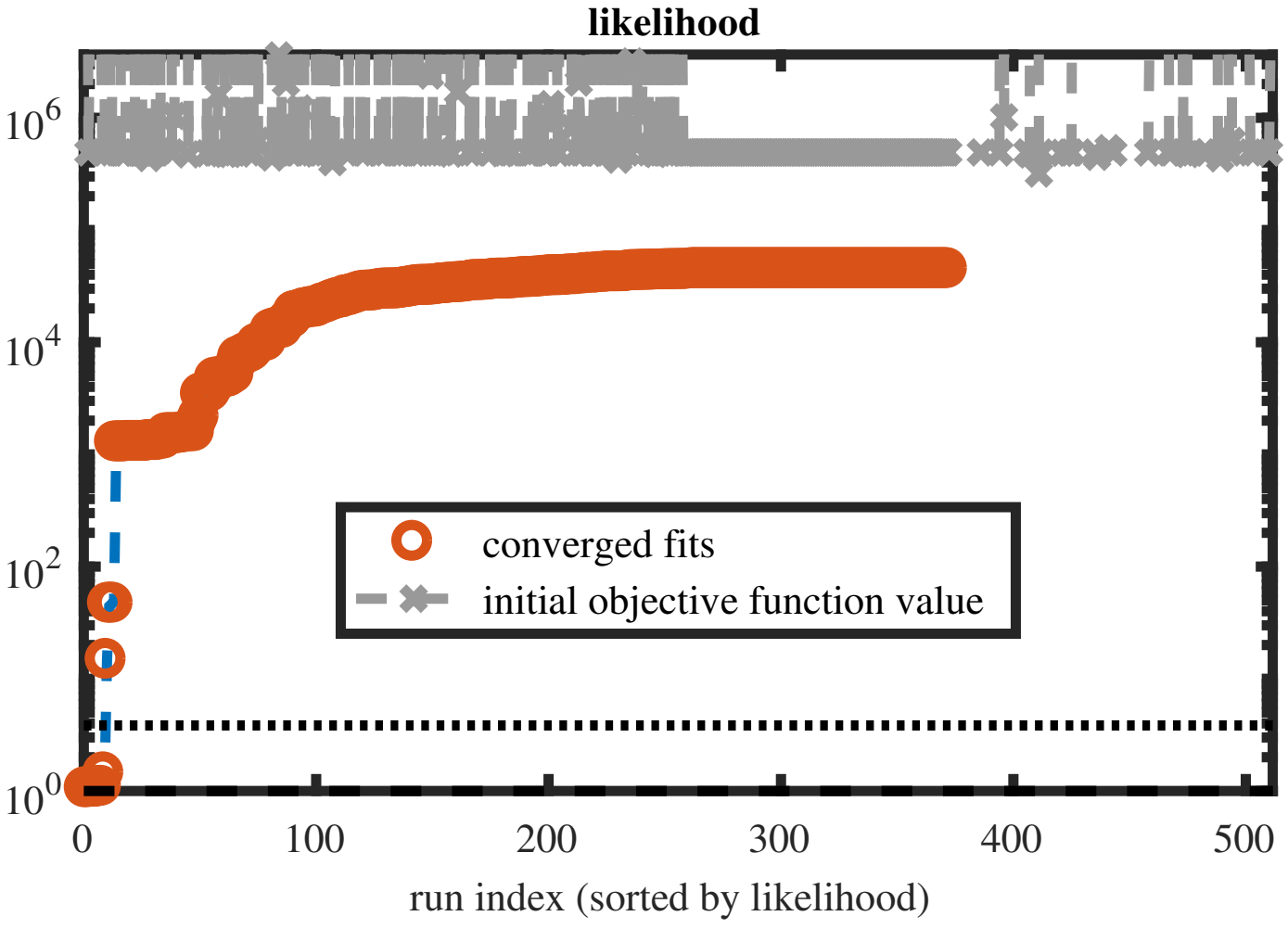
And "better" parameter identifiability



C3 is biased towards β-arr, compared to cAMP, in comparison to FSH.

With a "simpler" model

And "better" convergence curves



Summary

- Notion of signaling bias to quantify differential activation of several pathways by a Ligand at a given receptor.
- Standard quantification has several drawbacks (no time, limited to sigmoid scenario, et).
- We gave a kinetic interpretation of Ligand biased, which rely on kinetic data and dynamic (ODE) modeling, with numerical parameter estimation and L^1 penalization to reduce combinatorial complexity.

Summary

- Notion of signaling bias to quantify differential activation of several pathways by a Ligand at a given receptor.
- Standard quantification has several drawbacks (no time, limited to sigmoid scenario, et).
- We gave a kinetic interpretation of Ligand biased, which rely on kinetic data and dynamic (ODE) modeling, with numerical parameter estimation and L^1 penalization to reduce combinatorial complexity.

$\Rightarrow$ How to deal with "fuzzy/noisy" PLE ?

$\Rightarrow$ How to deal with non uniqueness of optimal parameters ?

$\Rightarrow$ How to perform a model reduction that would lead to both a satisfactory fit and identifiable parameters ?

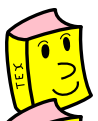
Thanks for your attention !

Bios Team, PRC, INRAE (Tours, Fr)

- ⋆ Eric Reiter
- ⋆ Pascale Crépieux
- ⋆ Anne Poupon
- ⋆ Frédéric Jean-Alphonse
- ⋆ Francesco De Pascali

United Arab Emirates University

- ⋆ Mohammed Ayoub



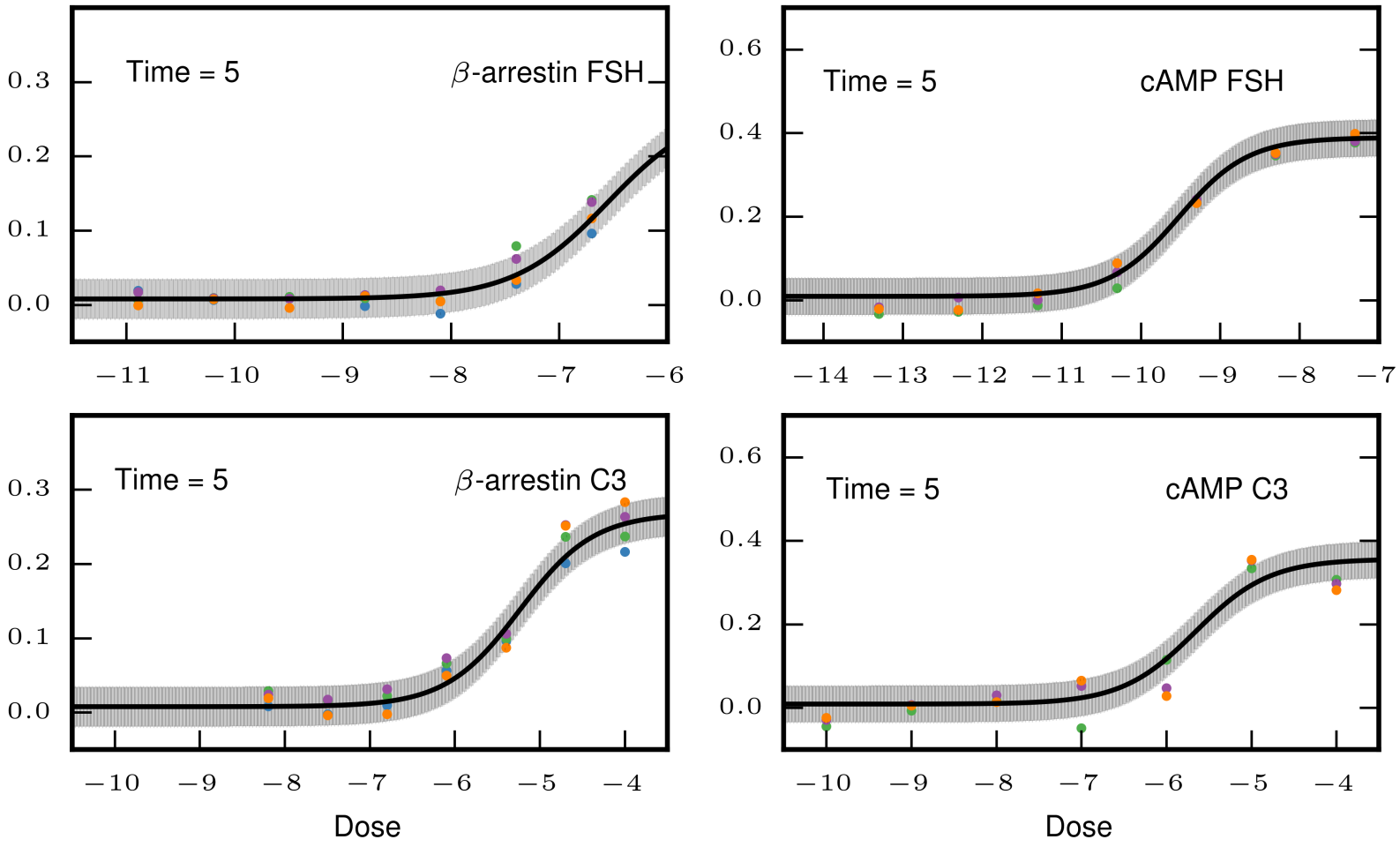
M. Ayoub et al., Molecular and Cellular Endocrinology 436 (2016)

L. Riccetti et al., Scientific Reports 7 :940 (2017)

R.Y. et al., Methods in Molecular Biology, in press (2018)

Comparison with dose-response (on FHSR in HEK cells)

We systematically calculate bias value using standard method (operational model on dose-response curves :)



Bias=2.3 : C3 is biased towards β-arr, compared to cAMP, in comparison to FSH.

Comparison with dose-response (on FHSR in HEK cells)

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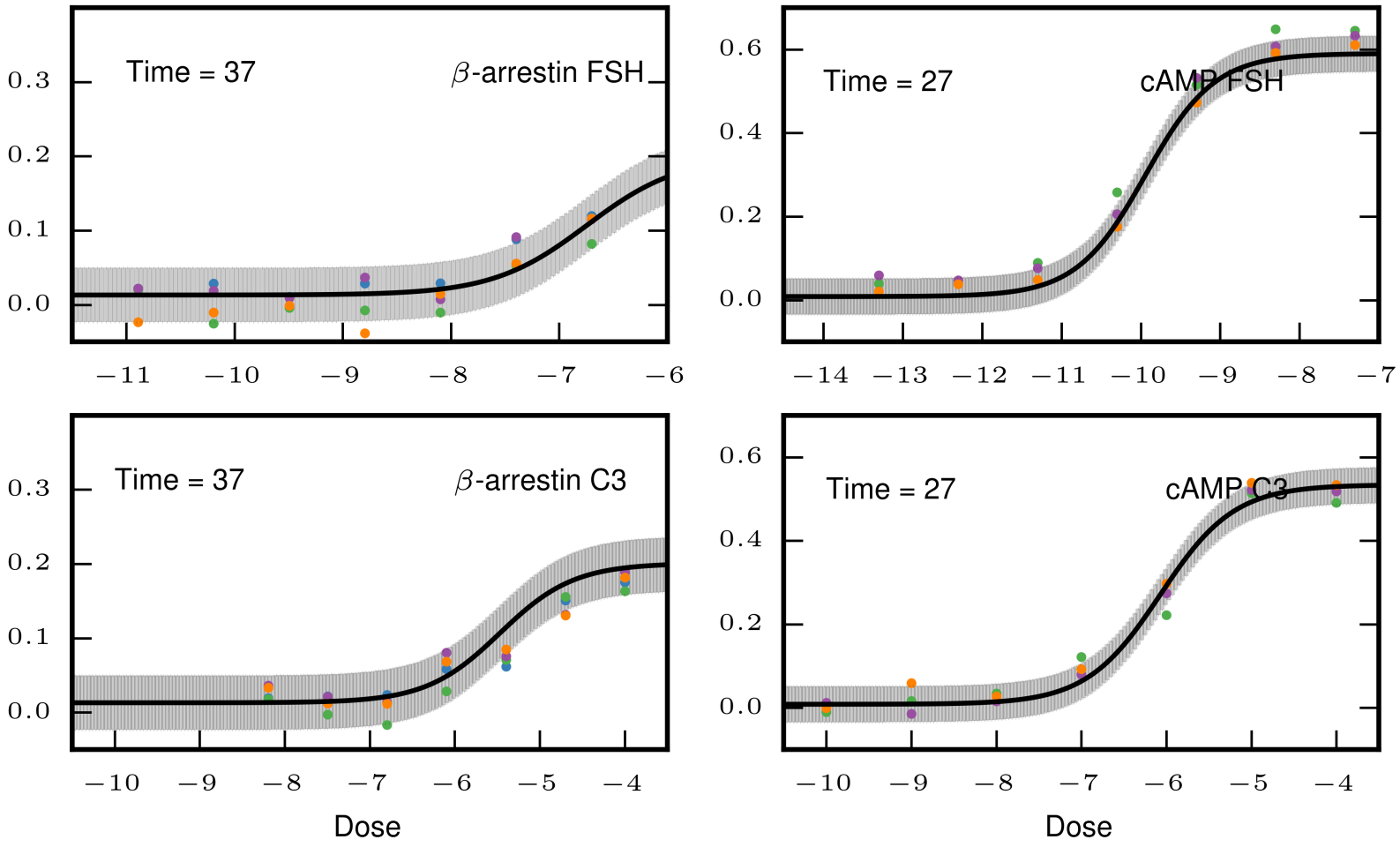

Bias=2.64 : C3 is biased towards β-arr, compared to cAMP, in comparison to FSH.

Comparison with dose-response (on FHSR in HEK cells)

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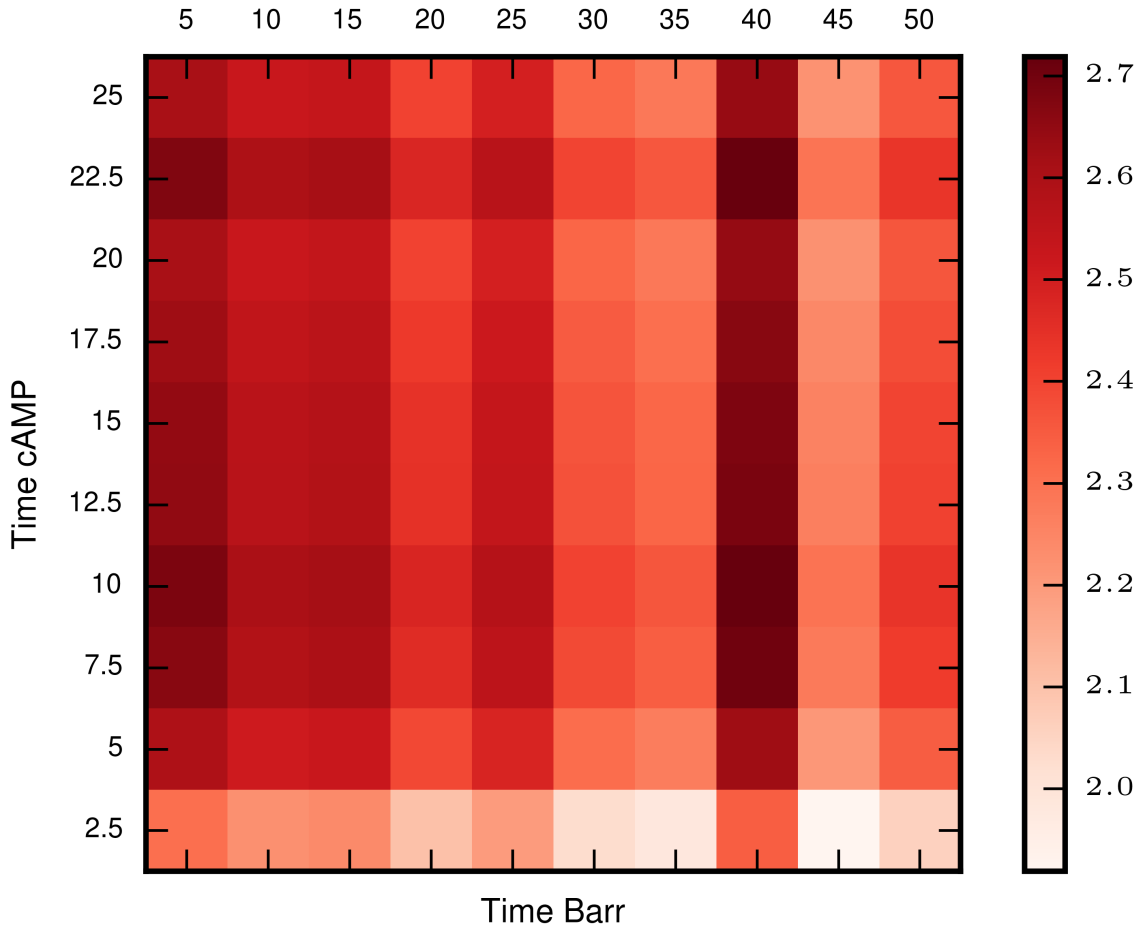
Different times gives (slightly) different bias values

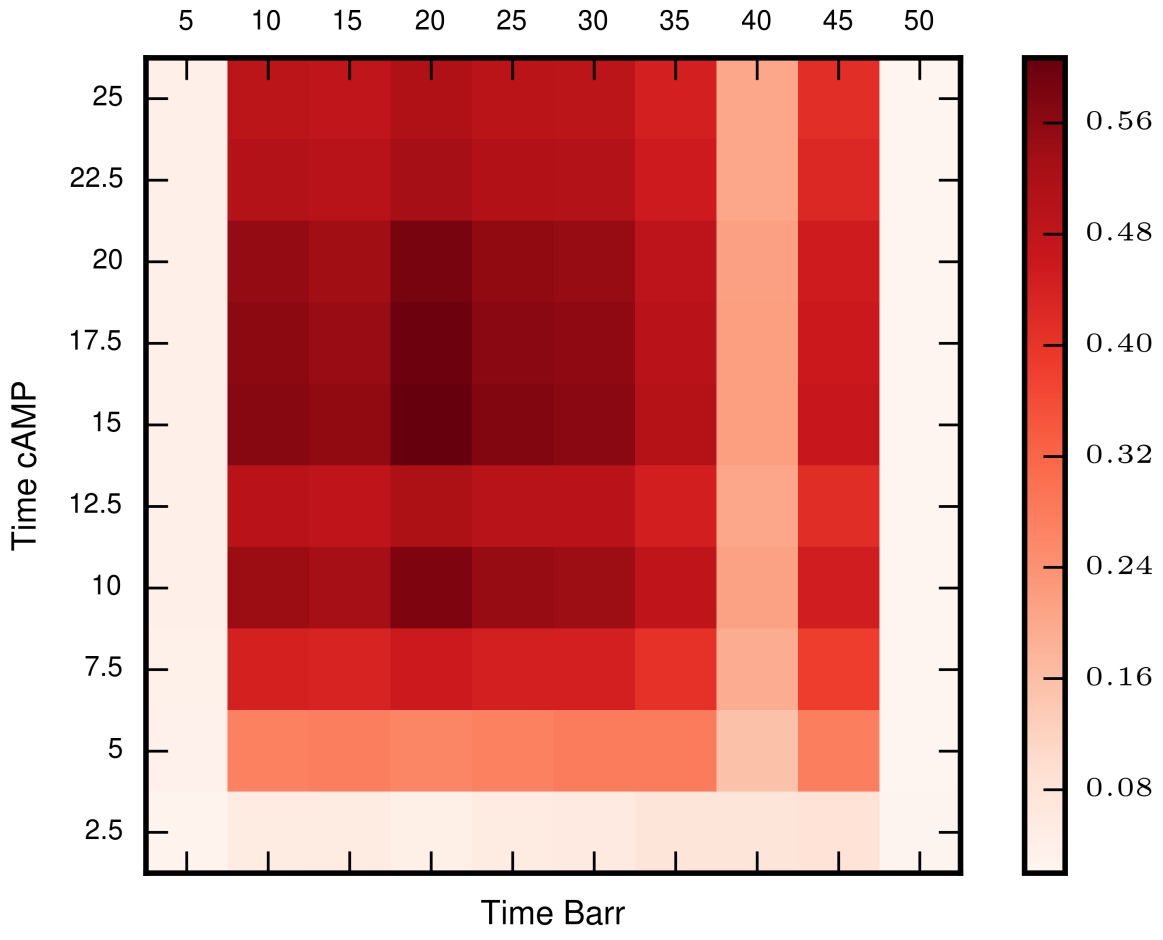


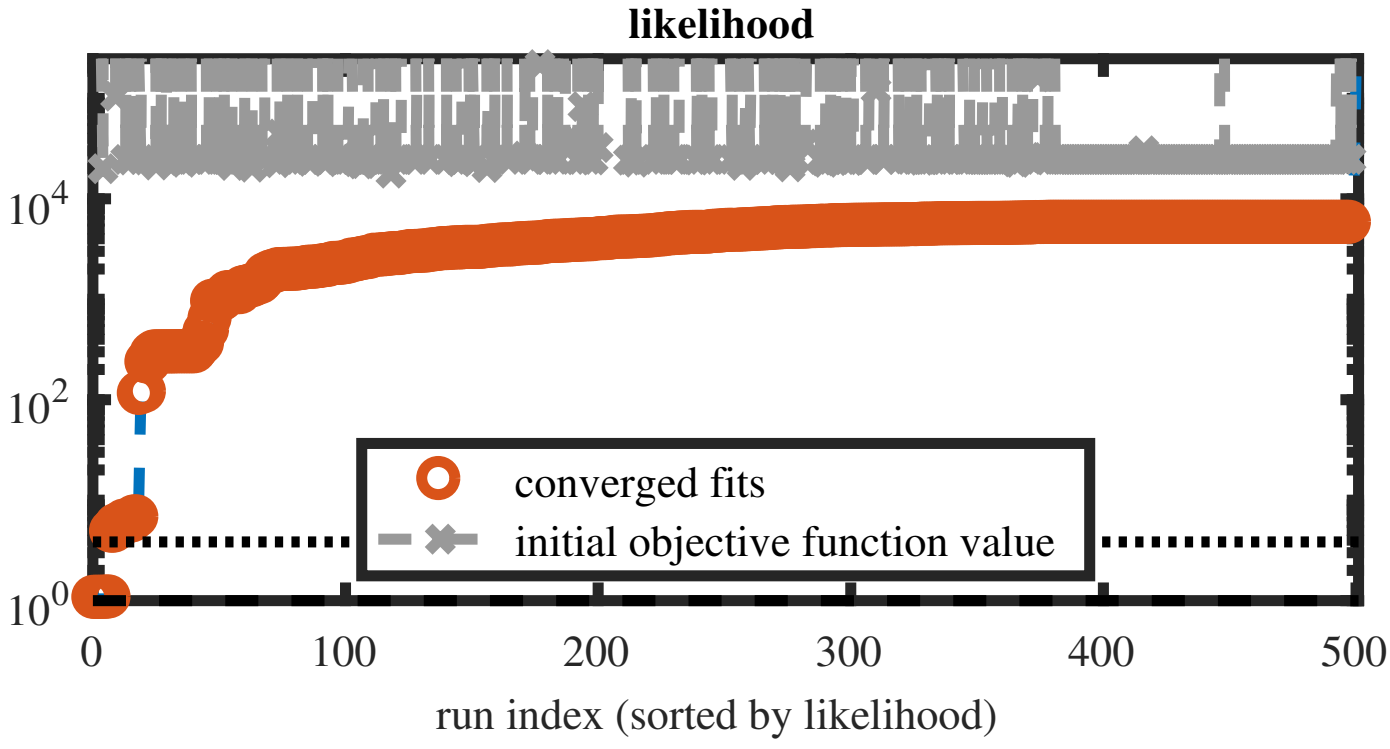
C3 is biased towards β-arr, compared to cAMP, in comparison to FSH.

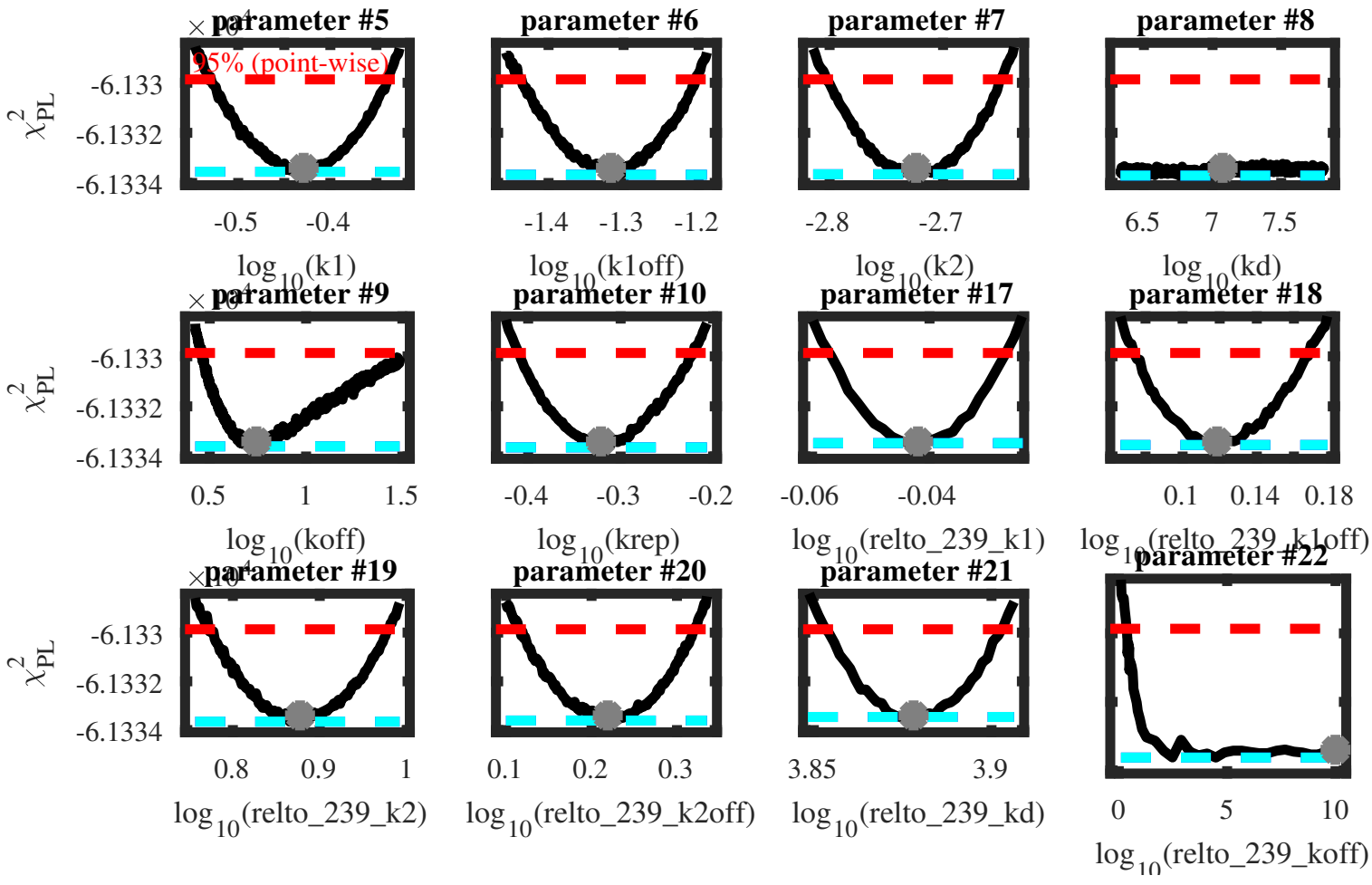
Comparison with dose-response (on FHSR in HEK cells)

We systematically calculate bias value using standard method

Uncertainty can be large according to the time of measurement

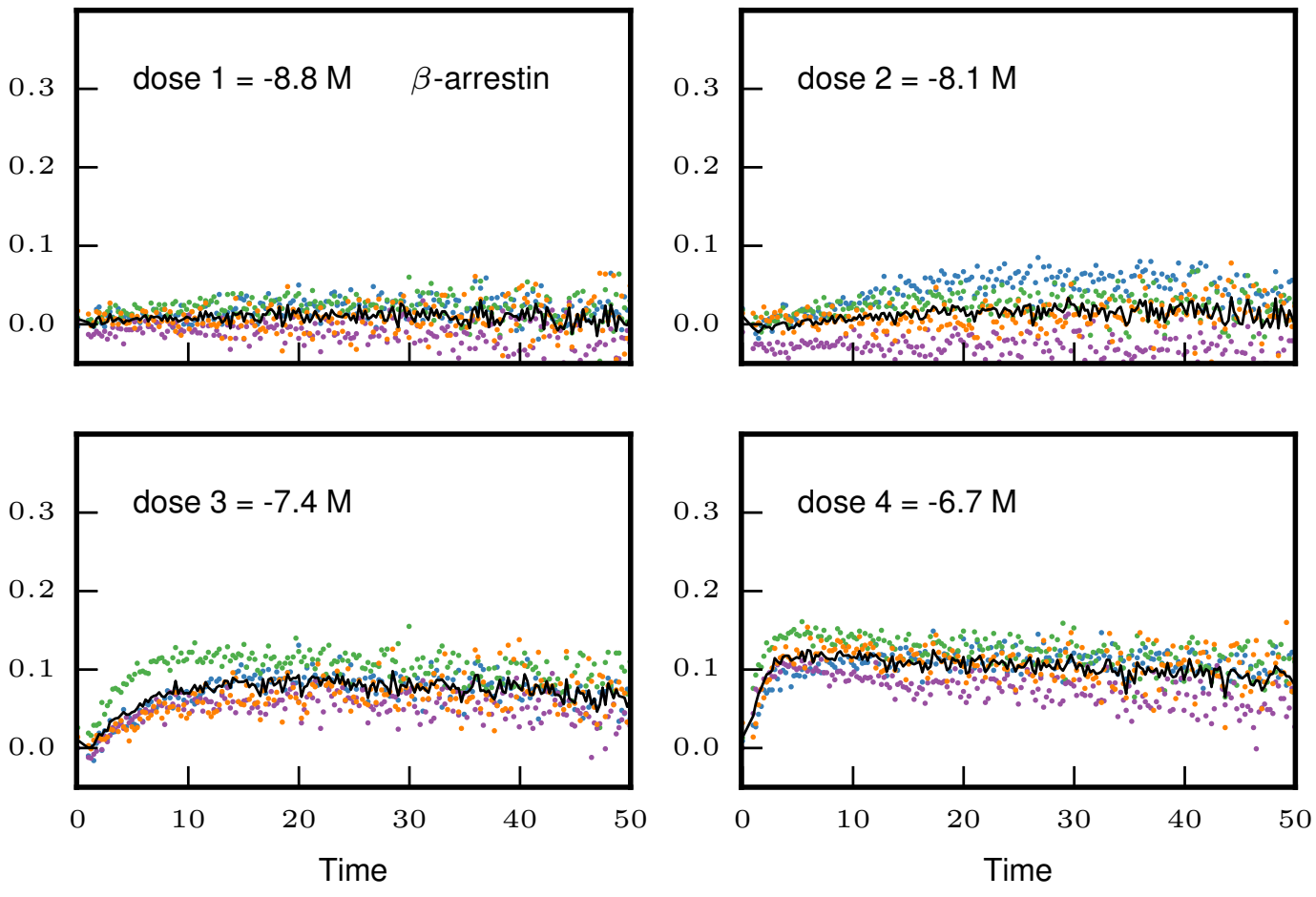



likelihood
10^4
10^2
10^0
converged fits
initial objective function value
0
100
200
300
400
500
run index (sorted by likelihood)


parameter #5
parameter #6
parameter #7
parameter #8
parameter #9
parameter #10
parameter #17
parameter #18
parameter #19
parameter #20
parameter #21
parameter #22
95% (point-wise)
χ^2_{PL}
-6.133
-6.1332
-6.1334
$\log_{10}$(k1)
$\log_{10}$(k1off)
$\log_{10}$(k2)
$\log_{10}$(kd)
$\log_{10}$(koff)
$\log_{10}$(krep)
$\log_{10}$(relto_239_k1)
$\log_{10}$(relto_239_k1off)
$\log_{10}$(relto_239_k2)
$\log_{10}$(relto_239_k2off)
$\log_{10}$(relto_239_kd)
$\log_{10}$(relto_239_koff)

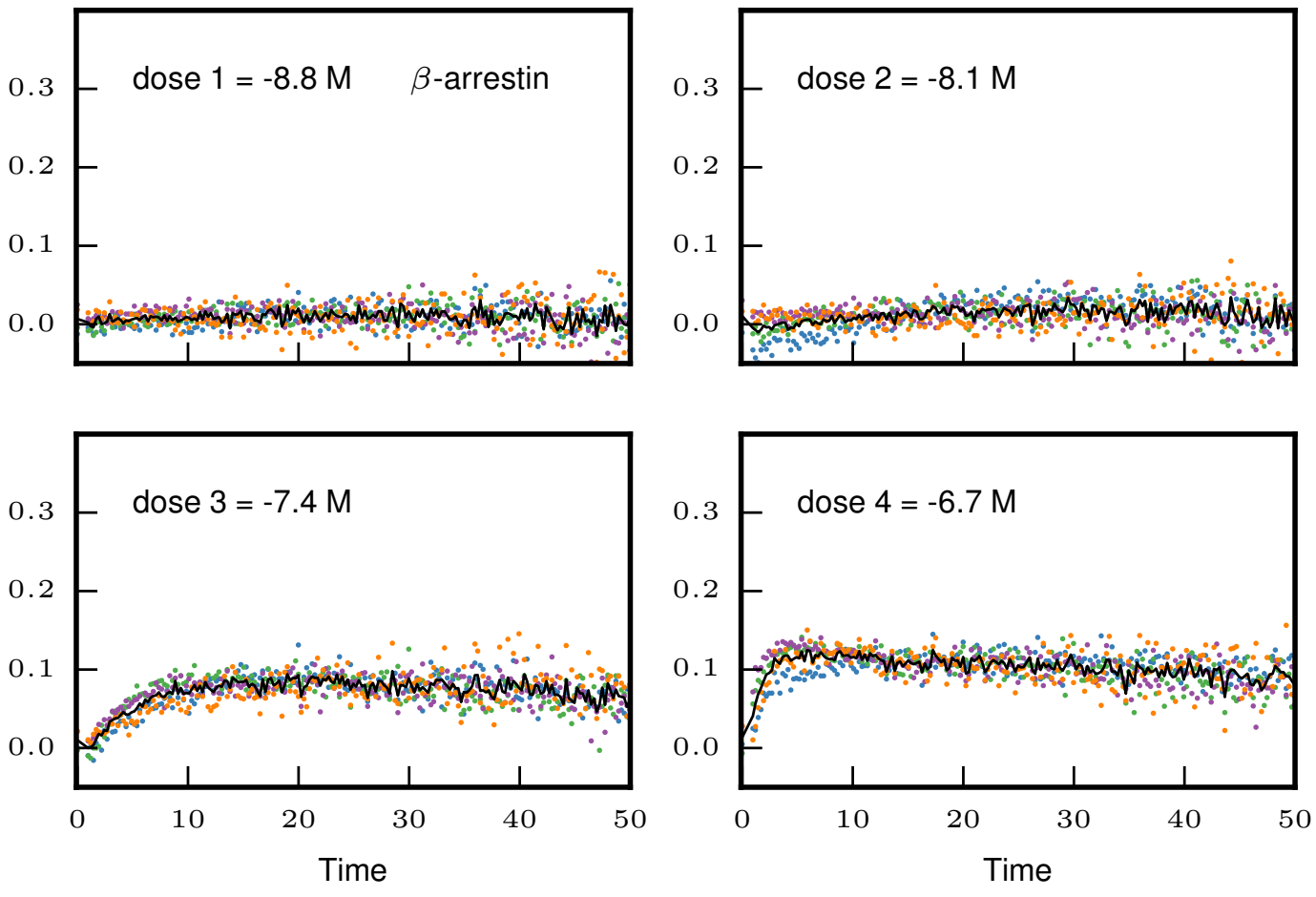
("trick" to minimize variance...)

Original "raw" data



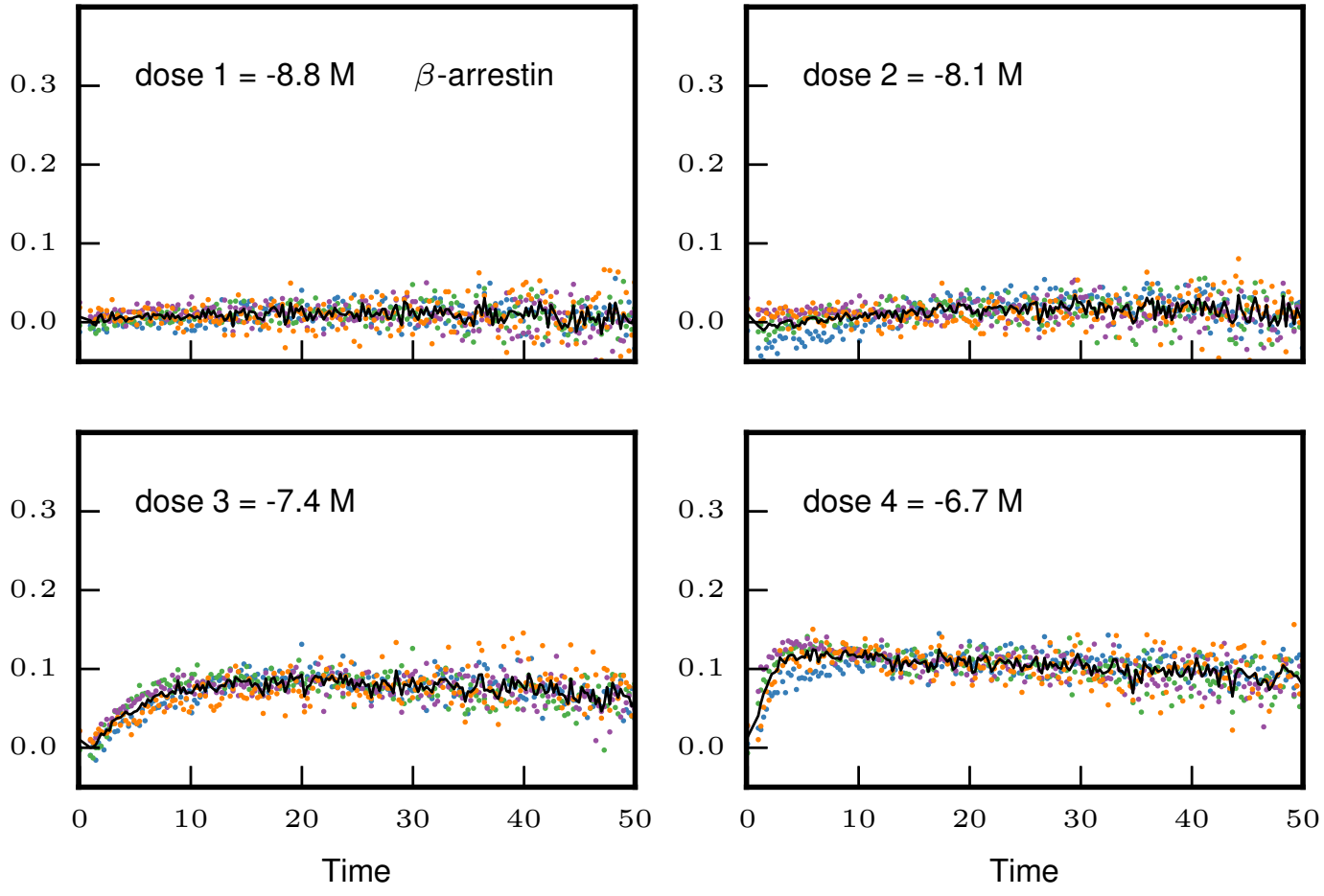
("trick" to minimize variance...)

"Adjusted" data



("trick" to minimize variance...)

"Adjusted" data



+ adjusting the number of data points ...

Other extensions

Dose-dependent bias

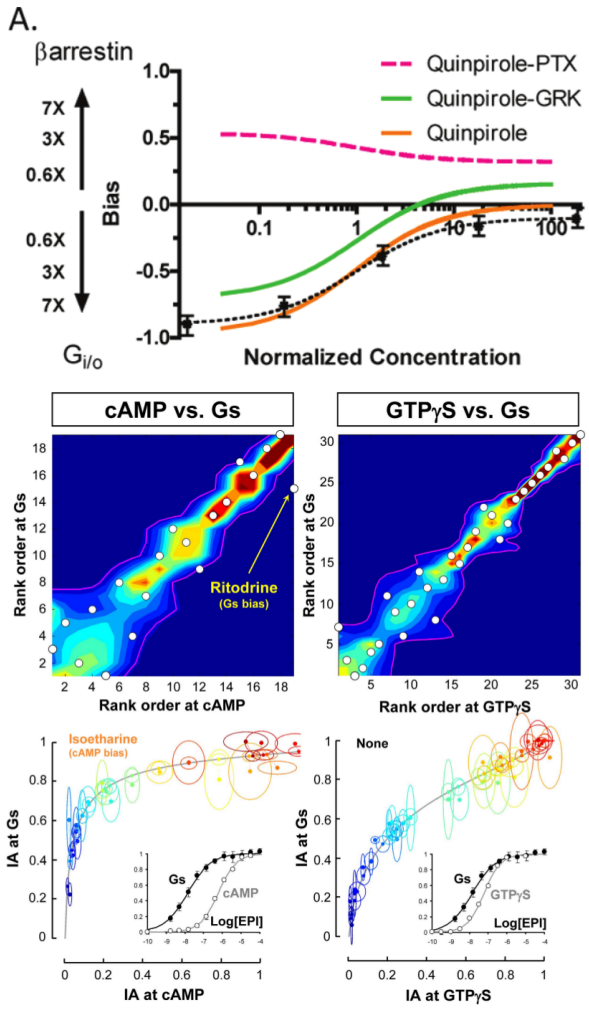
Barak and Peterson et al., *Biochem.* (2012)

Extension of the operational model

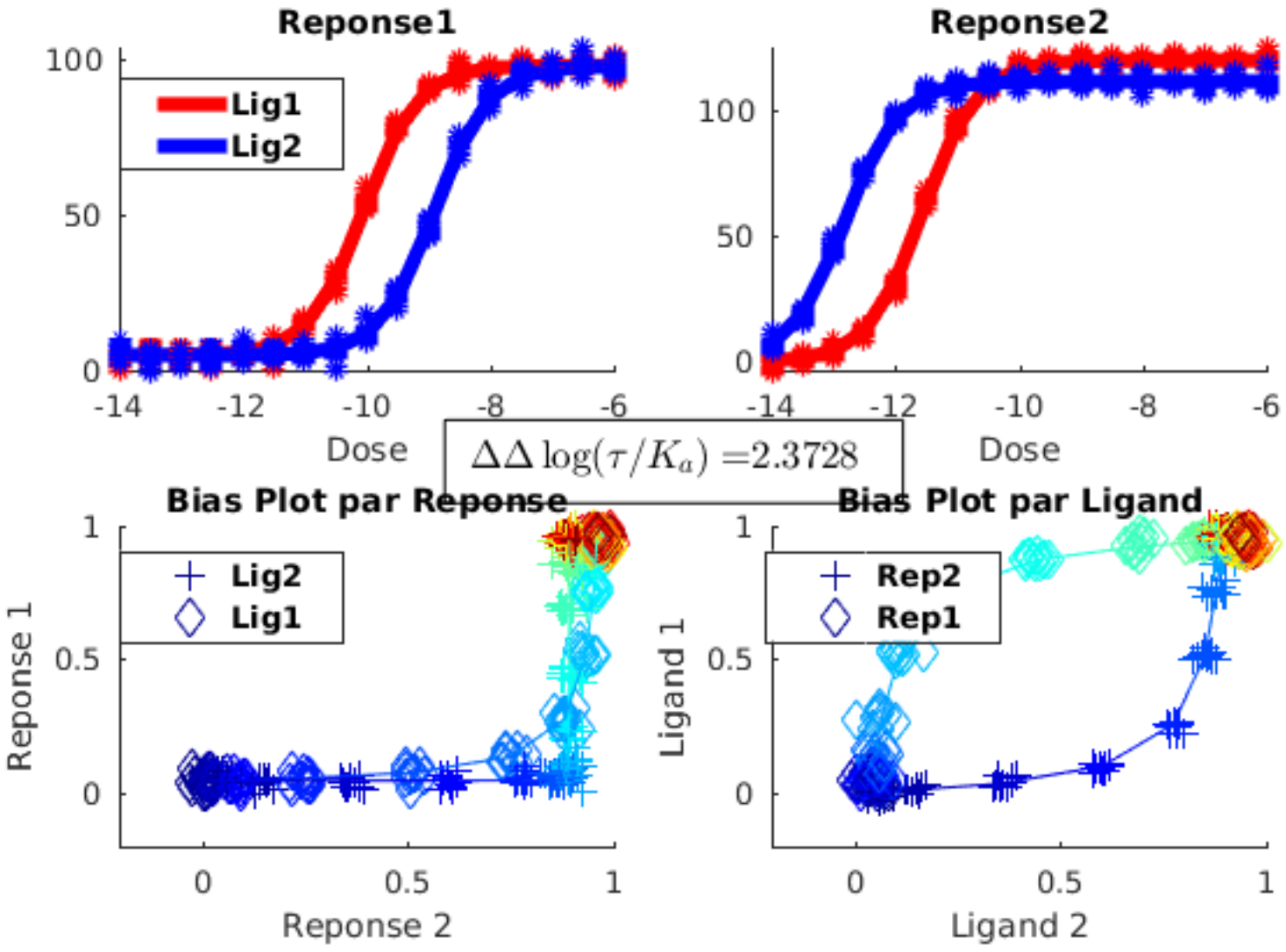
Kenakin, *Chem. Rev.* (2017)

Method based on Intrinsic activities and rank ordering

Onaran et al., *Sci. Rep.* (2017)

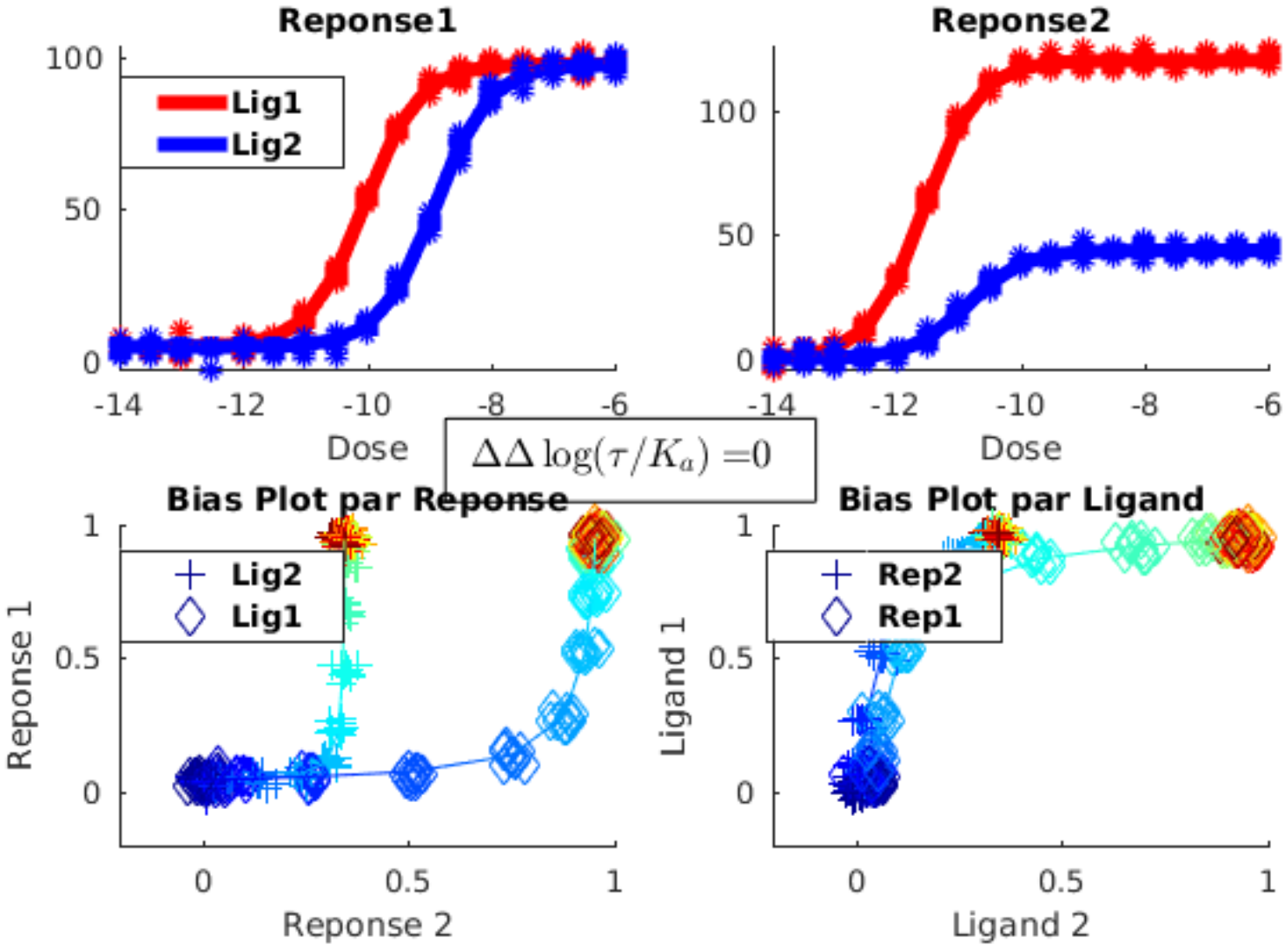


Is bias calculation intuitive ? (simulated data)



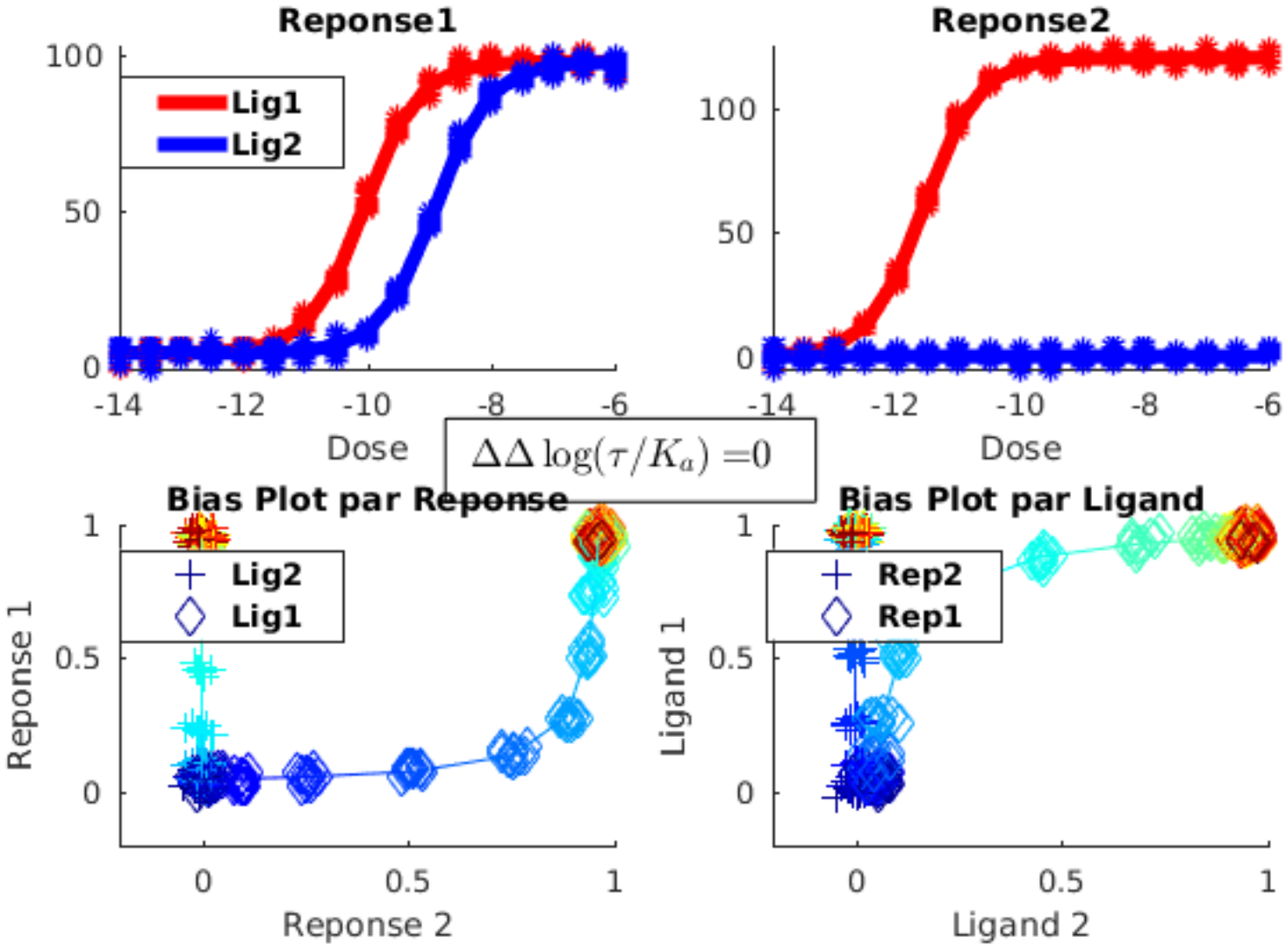
A strong bias is usually 'apparent' on dose-response curves or bias plot

Is bias calculation intuitive ? (simulated data)



But there may be counter-intuitive situation...

Is bias calculation intuitive ? (simulated data)



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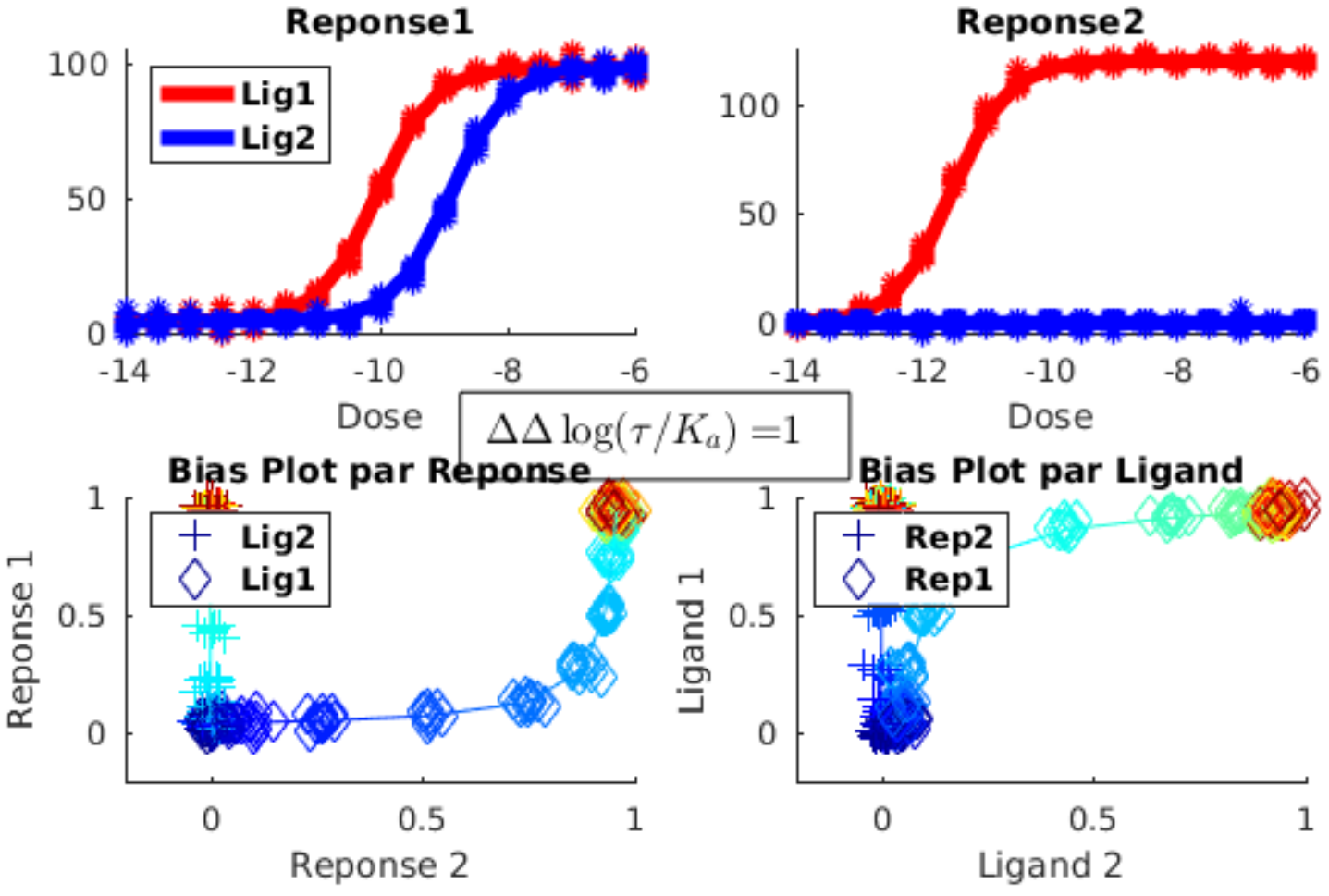

But there may be counter-intuitive situation...

... and those situations occur in real life !